Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/DK04/000880

International filing date: 17 December 2004 (17.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: DK
Number: PA 2003 01895
Filing date: 19 December 2003 (19.12.2003)

Date of receipt at the International Bureau: 14 January 2005 (14.01.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

PCT/DK 2004/000880

Kongeriget Danmark

Patent application No.:	PA 2003 01895
Date of filing:	19 December 2003
Applicant: (Name and address)	Novozymes A/S Krogshøjvej 36 DK-2880 Bagsværd Denmark

Title: Mashing process

IPC: -

This is to certify that the attached documents are exact copies of the above mentioned patent application as originally filed.

Patent- og Varemærkestyrelsen · Kongeriget Danmark

Patent- og Varemærkestyrelsen
Økonomi- og Erhvervsministeriet

07 January 2005

Susanne Morsing

PATENT- OG VAREMÆRKESTYRELSEN

MASHING PROCESS

FIELD OF THE INVENTION

The present invention relates, inter alia, to a mashing and filtration step in a process for the production of an alcoholic beverage, such as beer or whiskey, and to a composition useful in the mashing and filtration step in such a process.

BACKGROUND OF THE INVENTION

The use of enzymes in brewing is common. Application of enzymes to the mashing step to improve mash filterability and increase extract yield is described in WO 97/42302. However, there is a need for improvement of the mashing and filtration step and for improved enzymatic compositions for use in the mashing and filtration step.

SUMMARY OF THE INVENTION

The invention provides a process for production of a mash having enhanced filterability and/or improved extract yield after filtration, which comprises; preparing a mash in the presence of enzyme activities and filtering the mash to obtain a wort, wherein the enzyme activities comprise; a xylanase of glucoside hydrolase family 10 present in an amount of at least 15% w/w of the total xylanase and endoglucanase enzyme protein.

In a further aspect the invention provides a process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising: testing at least one xylanolytic enzyme for its hydrolytic activity towards insoluble wheat arabinoxylan, selecting a xylanolytic enzyme which cleaves next to branched residues thereby leaving terminal substituted xylose oligosaccharides, and adding the selected xylanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

In an even further aspect the invention provides a process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising: testing at least one endoglucanolytic enzyme for its hydrolytic activity towards barley beta-glucan, selecting a endoglucanolytic enzyme which under the conditions: 10 microgram/ml purified enzyme and 5 mg/ml barley beta-glucan in 50 mM sodium acetate, 0.01% Triton X-100, at pH 5.5 and 50°C, within 1 hour degrades more than 70% of the barley beta-glucan to DP 6 or DP<6, and adding the selected endoglucanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

In yet a further aspect the invention provides a composition comprising; a GH10 xylanase present in an amount of at least 15% w/w of the total enzyme protein; and/or, a GH12, GH7 and/or GH5 endoglucanase present in an amount of at least 40% w/w of the total enzyme protein.

Other aspects include the use of the composition of the proceeding aspect in a process of comprising reduction of the viscosity of an aqueous solution comprising a starch

hydrolysate, including such processes wherein the aqueous solution comprising a starch hydrolysate is a mash for beer making, or wherein the aqueous solution comprising a starch hydrolysate is intended for use in a feed composition.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Throughout this disclosure, various terms that are generally understood by those of ordinary skill in the arts are used. Several terms are used with specific meaning, however, and are meant as defined by the following.

As used herein the term "**grist**" is understood as the starch or sugar containing material that's the basis for beer production, e.g. the barley malt and the adjunct.

The term "**malt**" is understood as any malted cereal grain, in particular barley.

The term "**adjunct**" is understood as the part of the grist which is not barley malt. The adjunct may be any carbohydrate rich material.

The term "**mash**" is understood as a aqueous starch slurry, e.g. comprising crushed barley malt, crushed barley, and/or other adjunct or a combination hereof, steeped in water to make wort.

The term "**wort**" is understood as the unfermented liquor run-off following extracting the grist during mashing.

The term "**spent grains**" is understood as the drained solids remaining when the grist has been extracted and the wort separated from the mash.

The term "**beer**" is here understood as fermented wort, e.g. an alcoholic beverage brewed from barley malt, optionally adjunct and hops.

The term "**extract recovery**" in the wort is defined as the sum of soluble substances extracted from the grist (malt and adjuncts) expressed in percentage based on dry matter.

The term "**a thermostable enzyme**" is understood as an enzyme that under the temperature regime and the incubation period applied in the processes of the present invention in the amounts added is capable of sufficient degradation of the substrate in question.

The term "**Type A xylanase**" is understood as a xylanase that cleaves arabinoxylan polymers close to branched residues leaving terminal substituted xylose oligosaccharides. Type A xylanases may be identified using the method described in the Methods section of the present disclosure

The term "**homology**" when used about polypeptide or DNA sequences and referred to in this disclosure is understood as the degree of homology between two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the

GCG program package (Program Manual for the Wisconsin Package. Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453. The following settings for polypeptide sequence comparison are used: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

The term **"DP"** is the degree of polymerisation, herein used for average number of glucose units in polymers in a polysaccharide hydrolysate.

The numbering of **Glycoside Hydrolase Families** (GH) and **Carbohydrate Binding Modules** (CBM) applied in this disclosure follows the concept of Coutinho, P.M. & Henrissat, B. (1999) *CAZy - Carbohydrate-Active Enzymes server* at URL: http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html or alternatively Coutinho, P.M. & Henrissat, B. 1999; The modular structure of cellulases and other carbohydrate-active enzymes: an integrated database approach. In *"Genetics, Biochemistry and Ecology of Cellulose Degradation"*., K. Ohmiya, K. Hayashi, K. Sakka, Y. Kobayashi, S. Karita and T. Kimura eds., Uni Publishers Co., Tokyo, pp. 15-23, and in Bourne, Y. & Henrissat, B. 2001; Glycoside hydrolases and glycosyltransferases: families and functional modules, *Current Opinion in Structural Biology* 11:593-600. This classification system groups glucoside hydrolases based on similarities in primary structure. The members of a family furthermore show the same catalytic mechanism and have similarities in the overall three-dimensional structure, although a family may contain members with substantial variation in substrate specificity.

The naming of *Humicola insolens* endoglucanases follows the system of Karlsson, J. 2000. Fungal Cellulases, Study of hydrolytic properties of endoglucanases from *Trichoderma reesei* and *Humicola insolens*. Lund University.

Brewing processes are well-known in the art, and generally involve the steps of malting, mashing, and fermentation. In the traditional brewing process the malting serves the purpose of converting insoluble starch to soluble starch, reducing complex proteins, generating colour and flavour compounds, generating nutrients for yeast development, and the development of enzymes. The three main steps of the malting process are steeping, germination, and kilning.

Steeping includes mixing the barley kernels with water to raise the moisture level and activate the metabolic processes of the dormant kernel. In the next step, the wet barley is germinated by maintaining it at a suitable temperature and humidity level until adequate modification, i.e. such as degradation of starch and activation of enzymes, has been achieved. The final step is to dry the green malt in the kiln. The temperature regime in the kiln determines the colour of the barley malt and the amount of enzymes which survive for use in the mashing process. Low temperature kilning is more appropriate for malts when it is essential to preserve enzymatic activity. Malts kilned at high temperatures have very little or no enzyme activity but are very high in colouring such as caramelized sugars as well as in flavouring compounds.

Mashing is the process of converting starch from the milled barley malt and solid adjuncts into fermentable and unfermentable sugars to produce wort of the desired composition. Traditional mashing involves mixing milled barley malt and adjuncts with water at a set temperature and volume to continue the biochemical changes initiated during the malting process. The mashing process is conducted over a period of time at various temperatures in order to activate the endogenous malt enzymes responsible for the degradation of proteins and carbohydrates. By far the most important change brought about in mashing is the conversion of starch molecules into fermentable sugars. The principal enzymes responsible for starch conversion in a traditional mashing process are alpha- and beta-amylases. Alpha-amylase very rapidly reduces insoluble and soluble starch by splitting starch molecules into many shorter chains that can be attacked by beta-amylase. The disaccharide produced is maltose.

Traditionally lager beer has often been brewed using a method referred to as "step-infusion". This mashing procedure involves a series of rests at various temperatures, each favouring one of the necessary endogenous enzyme activities. To day the double-mash infusion system is the most widely used system for industrial production of beer, especially lager type beer. This system prepares two separate mashes. It utilizes a cereal cooker for boiling adjuncts and a mash tun for well-modified, highly enzymatically active malts.

When brewing from grists low in enzymes such as high adjunct grists, mashing may be performed in the presence of added enzyme compositions comprising the enzymes necessary for the hydrolysis of the grist starch. These enzymes may comprise alpha-amylases, pullulanases, beta-amylases and glucoamylases.

After mashing, it is necessary to separate the liquid extract (the wort) from the solids (spent grains i.e. the insoluble grain and husk material forming part of grist). Wort separation is important because the solids contain large amounts of non-starch polysaccharides, protein, poorly modified starch, fatty material, silicates, and polyphenols (tannins). Important non-starch polysaccharides present in cereal grains are beta-glucan and arabinoxylan. The endosperm cell wall of barley comprises 75% beta-glucan, 20% arabinoxylan, and 5% remaining protein with small amount of cellulose, glucomannan and phenolic acids. Long chains of barley arabinoxylans, and to a lesser degree beta-glucan, which have not been modified due to enzymatic hydrolysis may cause formation of gels when solubilised in water, these gels will strongly increase wort viscosity and reduce filterability. Likewise is it very important for the quality of the wort that the beta-glucan has been reduced to smaller oligomers, as unmodified beta-glucans later on will give rise to haze stability problems in the final beer. Therefore, enzymatic compositions comprising endoglucanases and xylanases, such as Ultraflo® or Viscozyme®, are often used in the mashing step to improve wort separation. The objectives of wort separation, *inter alia*, include the following:

- to obtain good extract recovery,

- to obtain good filterability, and
- to produce clear wort.

Extraction recovery and filterability are important for the economy in the brewing process, while the wort clarity is a must in order to produce a beer which does not develop haze. Extraction recovery, filterability and wort clarity is greatly affected by the standard of the grist, e.g. the barley malt and the types of adjunct, as well as the applied mashing procedure.

Following the separation of the wort from the spent grains the wort may be fermented with brewers yeast to produce a beer.

Further information on conventional brewing processes may be found in "Technology Brewing and Malting" by Wolfgang Kunze of the Research and Teaching Institute of Brewing, Berlin (VLB), 2nd revised Edition 1999, ISBN 3-921690-39-0.

Embodiments of the invention

The invention provides a process for production of a mash having enhanced filterability and/or improved extract yield after filtration, which comprises; preparing a mash in the presence of enzyme activities and filtering the mash to obtain a wort, wherein the enzyme activities comprise; a xylanase of GH family 10 present in an amount of at least 15%, 20%, preferably 25%, such as at least 30%, or at least 40%, at least 50% or at least 60% such as at least 70%, at least 80%, at least 90%, or even 100% w/w of the total xylanase and endoglucanase enzyme protein.

In a preferred embodiment the xylanase is a type A xylanase, and in a particular embodiment the xylanase is a type A xylanase having a $I_{1,3terminal}/I_{1,3internal}$ ratio of at least 0.25, such as at least 0.30, al least 0.40, at least 0.50, or even at least 0.60.

Preferably the xylanase has a CBM, preferably a CBM of family 1.

In another preferred embodiment the xylanase is a xylanase which in the xylanase binding assay described herein has a barley soluble/insoluble fibre binding ratio of at least 0.50, preferably at least 0.60, more preferably at least 0.70, such as 0.80, 0.90, 1.00, 1.10 or even at least 1.20.

In another preferred embodiment the xylanase is derived from a filamentous fungi such as from a strain of an *Aspergillus* sp., preferably from *Aspergillus aculeatus* (SEQ ID NO:8 or SEQ ID NO:9), from a strain of a *Myceliophotora* sp., preferably from a *Myceliophotora thermophilia* (SEQ ID NO:13), from a strain of a *Humicola* sp., preferably from *Humicola insolens* (SEQ ID NO:12). In a more preferred embodiment the xylanase has at least 50%, such as at least 60%, 70%, 80% or even 90% homology to any of the aforementioned sequences.

In another preferred embodiment the xylanase is derived from a bacterium such as from a strain of a *Bacillus*, preferably from *Bacillus halodurans*.

1. In another preferred embodiment the endoglucanase is an endoglucanase derived from *Humicola*

sp., such as the endoglucanase from *Humicola insolens* (SEQ ID NO:3), or the endoglucanase from *H. insolens* (SEQ ID NO:4), from *Thermoascus* sp., such as the endoglucanase derived from *Thermoascus aurantiacus* (SEQ ID NO:6), or from *Aspergillus* sp., such as the endoglucanase derived from *Aspergillus aculeatus* (SEQ ID NO:16).

In a preferred embodiment the xylanase has at least 50%, such as at least 60%, 70%, 80% or even 90% homology to any of the aforementioned sequences.

In another preferred embodiment at least one additional enzyme is present, which enzyme is arabinofuranosidase.

The invention also provides a process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising: testing at least one xylanolytic enzyme for its hydrolytic activity towards insoluble wheat arabinoxylan, selecting a xylanolytic enzyme which cleaves next to branched residues thereby leaving terminal substituted xylose oligosaccharides, and adding the selected xylanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

The invention further provides a process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising: testing at least one endoglucanolytic enzyme for its hydrolytic activity towards barley beta-glucan, selecting a endoglucanolytic enzyme which under the conditions: 10 microgram/ml purified enzyme and 5 mg/ml barley beta-glucan in 50 mM sodium acetate, 0.01% Triton X-100, at pH 5.5 and 50°C, within 1 hour degrades more than 70% of the barley beta-glucan to DP 6 or DP<6, and adding the selected endoglucanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

In preferred embodiments of the two processes the aqueous solution comprising a starch hydrolysate is a mash for beer making.

The invention also provides a composition comprising; a GH10 xylanase present in an amount of at least 15% w/w of the total enzyme protein; and/or, a GH12, GH7 and/or GH5 endoglucanase present in an amount of at least 40% w/w of the total enzyme protein.

In a preferred embodiment the xylanase of the composition is a type A xylanase, and preferably a type A xylanase having a $I_{1,3terminal}/I_{1,3internal}$ ratio of at least 0.25, such as at least 0.30, at least 0.40, at least 0.50, or even at least 0.60.

In a preferred embodiment the xylanase of the composition is derived from a filamentous fungi such as from a strain of an *Aspergillus* sp., preferably from *Aspergillus aculeatus* (SEQ ID NO:8 or SEQ ID NO:9), from a strain of a *Myceliophotora* sp., preferably from a *Myceliophotora thermophilia* (SEQ ID NO:13), from a strain of a *Humicola* sp., preferably from *Humicola insolens* (SEQ ID NO:12). In a preferred embodiment the xylanase of the composition has at least 50%, such as at least 60%, 70%, 80% or even 90% homology to any of the aforementioned sequences.

In a preferred embodiment the xylanase of the composition is derived from a bacterium such as from a strain of a *Bacillus*, preferably from *Bacillus halodurans*.

In a preferred embodiment the endoglucanase of the composition is endoglucanase is endoglucanase derived from *Humicola* sp., such as the endoglucanase from *Humicola insolens* (SEQ ID NO:3), the endoglucanase from *H. insolens* (SEQ ID NO:4) or from *Thermoascus* sp., such as the endoglucanase derived from *Thermoascus aurantiacus* (SEQ ID NO:6), or from *Aspergillus* sp., such as the endoglucanase derived from *Aspergillus aculeatus* (SEQ ID NO:16).

. In a preferred embodiment the endoglucanase of the composition has at least 50%, such as at least 60%, 70%, 80% or even 90% homology to any of the aforementioned sequences.

In a preferred embodiment the xylanase GH family 10 of the composition is present in an amount of at least 20%, preferably at least 25%, such as at least 30%, at least 35%, at least 40%, at least 45% or even at least 50% w/w of the total xylanase and endoglucanase enzyme protein.

In a preferred embodiment the endoglucanase of GH Family 12, 7 and/or 5 endoglucanase of the composition is present in an amount of at least 25%, preferably 30%, such as at least 35%, at least 40%, at least 45% or even at least 50%, such as at least 55%, or even at least 60% w/w of the total xylanase and endoglucanase enzyme protein.

The composition according to the proceeding aspect may be used in a process comprising reducing the viscosity of an aqueous solution comprising a starch hydrolysate.

The composition may even be used in a process comprising filtering of an aqueous solution comprising a starch hydrolysate. In a preferred embodiment the aqueous solution comprising a starch hydrolysate is a mash for beer making, and in another preferred embodiment the aqueous solution comprising a starch hydrolysate is a feed composition.

The process of the invention may be applied in the mashing of any grist. According to the invention the grist may comprise any starch and/or sugar containing plant material derivable from any plant and plant part, including tubers, roots, stems, leaves and seeds. Preferably the grist comprises grain, such as grain from barley, wheat, rye, oat, corn, rice, milo, millet and sorghum, and more preferably, at least 10%, or more preferably at least 15%, even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w) of the grist of the wort is derived from grain. Most preferably the grist comprises malted grain, such as barley malt. Preferably, at least 10%, or more preferably at least 15%, even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w) of the grist of the wort is derived from malted grain.

For mashing of low malt grists the mashing enzymes may be exogenously supplied. The enzymes mostly used as starch degrading enzymes include pullulanases, alpha-amylases and amyloglucosidases. The use of starch degrading enzymes in mashing is well-known to the skilled person.

Adjunct comprising readily fermentable carbohydrates such as sugars or syrups may be added to the malt mash before, during or after the mashing process of the invention but is preferably added after the mashing process. A part of the adjunct may be treated with a protease and/or a endoglucanase, and/or heat treated before being added to the mash of the invention.

During the mashing process, starch extracted from the grist is gradually hydrolyzed into fermentable sugars and smaller dextrins. Preferably the mash is starch negative to iodine testing, before wort separation.

The application of the appropriate xylanase and endoglucanase activities in the process of the present invention results in efficient reduction of beta-glucan and arabino-xylan level facilitating wort separation, thus ensuring reduced cycle time, high extract recovery and clear wort.

The wort produced by the process of the first aspect of the invention may be fermented to produce a beer. Fermentation of the wort may include pitching the wort with a yeast slurry comprising fresh yeast, i.e. yeast not previously used for the invention or the yeast may be recycled yeast. The yeast applied may be any yeast suitable for beer brewing, especially yeasts selected from *Saccharomyces* spp. such as *S. cerevisiae* and *S. uvarum*, including natural or artificially produced variants of these organisms. The methods for fermentation of wort for production of beer are well known to the person skilled in the arts.

The process of the invention may include adding silica hydrogel to the fermented wort to increase the colloidal stability of the beer. The processes may further include adding kieselguhr to the fermented wort and filtering to render the beer bright. The beer produced by fermenting the wort of the invention may be any type of beer, e.g. ale, strong ale, stout, porter, lager, pilsner, bitter, export beer, malt liquor, happoushu, lambic, barley wine, high-alcohol beer, low-alcohol beer, low-calorie beer or light beer.

The beer produced by the process of the invention may be distilled to recover ethanol, e.g. for whisky production. Contemplated are any kind of whisky (spelled "whiskey" in US and Ireland) include bourbon, Canadian whisky, Irish whiskey, rye, and scotch.

Xylanase

For the present purposes a xylanase is an enzyme classified as EC 3.2.1.8. The official name is endo-1,4-beta-xylanase. The systematic name is 1,4-beta-D-xylan xylanohydrolase. Other names may be used, such as endo-(1-4)-beta-xylanase; (1-4)-beta-xylan 4-xylanohydrolase; endo-1,4-xylanase; xylanase; beta-1,4-xylanase; endo-1,4-xylanase; endo-beta-1,4-xylanase; endo-1,4-beta-D-xylanase; 1,4-beta-xylan xylanohydrolase; beta-xylanase; beta-1,4-xylan xylanohydrolase; endo-1,4-beta-xylanase; beta-D-xylanase. The reaction catalysed is the endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans.

While the xylanase to be used for the present invention may be of any origin including mammalian, plant or animal origin it is presently preferred that the xylanase is of microbial origin. In particular the xylanase may be one derivable from a filamentous fungus or a yeast.

Xylanases have been found in a number of fungal species, in particular species of *Aspergillus*, such as *A. niger, A. awamori, A. aculeatus and A. oryzae*, *Trichoderma*, such as *T. reesei* or *T. harzianum*, *Penicillium*, such as *P. camenbertii*, *Fusarium*, such as *F. oxysporum*, *Humicola*, such as *H. insolens*, and *Thermomyces lanuginosa*, such as *T. lanuginosa*. Xylanases have also been found in bacterial species, e.g. within the genus *Bacillus*, such as *B. pumilus*.

Preferably, according to the process of the invention the xylanase is derived from a filamentous fungus such as from *Aspergillus* sp., *Bacillus sp.*, *Humicola* sp., *Myceliophotora* sp., *Poitrasia* sp. or *Rhizomucor* sp.

Substrate specificity was shown to be a key parameter for the performance of xylanases in the process of the invention. A xylanase with optimum performance in the process of the invention seems to be an enzyme which binds rather strongly to soluble arabino-xylan and rather weakly to insoluble arabino-xylan. Preferably the xylanase to be used in the present invention is a xylanase which in the binding assay in the Methods description of this disclosure has a barley soluble/insoluble fibre binding ratio of at least 0.50, preferably at least 0.60, more preferably at least 0.70, such as 0.80, 0.90, 1.00, 1.10 or even at least 1.20.

A number of xylanases identified having these characteristics are members of the glucoside hydrolase family 10. Preferably the xylanase to be used in the present invention is a Glycoside Hydrolase Family 10 (GH10) xylanase, and most preferably the xylanase is a GH10 xylanase which is also a type A xylanase i.e. a xylanase which cleaves insoluble wheat arabinoxylan polymers close to branched residues leaving terminal substituted xylose oligosaccharides (please see the examples for a definition of type A and B). As the GH10 enzymes are able to go closer to the branched xylose units, they form smaller oligosaccharides than the GH11 xylanases.

Preferably the xylanase to be used in the present invention has a functional CBM, such as a CBM of family 1.

Preferably, according to the process of the invention the xylanase is selected from the list consisting of the xylanase from shown as, the xylanase from *Aspergillus aculeatus* shown as SEQ ID NO:8 (AA XYL I), the xylanase from *Aspergillus aculeatus* shown as SEQ ID NO:9 (AA XYL II), the xylanase from *Bacillus halodurans* shown as SWISS PROT P07528 (BH XYL A), the xylanase from *Humicola insolens* shown as SEQ ID NO:12 (HI XYL III), the xylanase from *Myceliophotora thermophila* shown as SEQ ID NO:13 (MT XYL I). Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned xylanase sequences.

Endoglucanase

For the present purposes an endoglucanase is an enzyme classified as EC 3.2.1.4. While the endoglucanase to be used for the present invention may be of any origin including mammalian, plant or animal origin it is presently preferred that the endoglucanase is of microbial origin. In particular the endoglucanase may be one derivable from a filamentous fungus or a yeast.

Preferably the endoglucanase is a Glycoside Hydrolase Family 12 (GH12), Glycoside Hydrolase Family 7 (GH7) or a Glycoside Hydrolase Family 5 (GH5) glucanase. More preferably the endoglucanase is a polypeptide having a beta-jelly-roll or a b8/a8-barrell in superstructure.

While the endoglucanase to be used for the present invention may be of any origin including mammalian, plant or animal origin it is presently preferred that the endoglucanase is of microbial origin. In particular the endoglucanase may be one derivable from a filamentous fungus or a yeast.

More preferably, according to the process of the invention the endoglucanase is derived from a filamentous fungus such as from *Aspergillus* sp. or *Humicola* sp.

Preferably, according to the process of the invention the endoglucanase is selected from the list consisting of the endoglucanase from *Aspergillus aculeatus* shown in SEQ ID NO:1 (AA EG I), the endoglucanase from *Aspergillus aculeatus* shown in SEQ ID NO:2 (AA EG II), the endoglucanase from *Aspergillus aculeatus* shown in SEQ ID NO:16 (AA EG III), the endoglucanase from *Humicola insolens* shown in SEQ ID NO:3 (HI EG I), the endoglucanase from *Humicola insolens* shown in SEQ ID NO:4 (HI EG III), the endoglucanase from *Humicola insolens* shown in SEQ ID NO:5 (HI EG IV). Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned sequences.

Other GH12 glucanases includes endoglucanases obtained from *Aspergillus* sp. such as from *Aspergillus kawachii* (SWISSPROT Q12679), or *Aspergillus niger* (SWISSPROT O74705), *Aspergillus oryzae* (SWISSPROT O13454), from *Erwinia* sp., such as from *Erwinia carotovora* (SWISSPROT P16630), and from *Thermotoga* sp., such as from *Thermotoga maritima* (SWISSPROT Q60032 or Q9S5X8). Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned GH12 glucanases sequences.

Other GH7 glucanases includes endoglucanases obtained from *Agaricus* sp., such as from *Agaricus bisporus* (SWISSPROT Q92400), from *Aspergillus* sp., such as from *Aspergillus niger* (SWISSPROT Q9UVS8), from *Fusarium* sp., such as from *Fusarium oxysporum* (SWISSPROT P46238), from *Neurospora* sp., such as from *Neurospora crassa* (SWISSPROT P38676), and from *Trichoderma* sp., such as from *Trichoderma longibrachiatum* (SWISSPROT Q12714). Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned GH7 glucanases sequences.

Other GH5 glucanases includes endoglucanases obtained from *Acidothermus* sp., such as from *Acidothermus cellulolyticus* (SWISSPROT P54583), from *Aspergillus* sp., such as from *Aspergillus niger* (SWISSPROT O74706), and from *Bacillus* sp., such as from *Bacillus polymyxa* (SWISSPROT P23548). Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned GH5 glucanases sequences.

Arabinofuranosidase

Arabinofuranosidase EC 3.2.1.55, common name alpha-N-arabinofuranosidase hydrolysise terminal non-reducing alpha-L-arabinofuranoside residues in alpha-L-arabinosides. The enzyme acts on alpha-L-arabinofuranosides, alpha-L-arabinans containing (1,3)- and/or (1,5)-linkages, arabinoxylans and arabinogalactans.

MATERIALS AND METHODS

Xylanase activity

The xylanolytic activity can be expressed in FXU-units, determined at pH 6.0 with remazol-xylan (4-O-methyl-D-glucurono-D-xylan dyed with Remazol Brilliant Blue R, Fluka) as substrate.

A xylanase sample is incubated with the remazol-xylan substrate. The background of non-degraded dyed substrate is precipitated by ethanol. The remaining blue colour in the supernatant (as determined spectrophotometrically at 585 nm) is proportional to the xylanase activity, and the xylanase units are then determined relatively to an enzyme standard at standard reaction conditions, i.e. at 50.0 °C, pH 6.0, and 30 minutes reaction time.

A folder AF 293.6/1 describing this analytical method in more detail is available upon request to Novozymes A/S, Denmark, which folder is hereby included by reference.

Glucanase activity

The cellulytic activity may be measured in fungal endoglucanase units (FBG), determined on a 0.5% beta-glucan substrate at 30°C, pH 5.0 and reaction time 30 min. Fungal endoglucanase reacts with beta-glucan releases glucose or reducing carbohydrate which is determined as reducing sugar according to the Somogyi-Nelson method.

1 fungal endoglucanase unit (FBG) is the amount of enzyme which according to the above outlined standard conditions, releases glucose or reducing carbohydrate with a reduction capacity equivalent to 1 micromol glucose per minute.

Enzymes

Ultraflo® L, a multicomponent enzyme composition derived from *Humicola insolens* comprising a mixture of endoglucanases, xylanases, pentosanases and arabanases.

Ultraflo® L is standardized to 45 FBG/g, and has a gravity of approximately 1.2 g/ml. Ultraflo® is available from Novozymes A/S.

Viscozyme® L, a multicomponent enzyme composition derived from *Aspergillus aculeatus* comprising a mixture of endoglucanases, arabanases and xylanases. Viscozyme® L is standardized to 100 FBG/g, and has a gravity of approximately 1.2 g/m l. Viscozyme is available from Novozymes A/S.

Alcalase®, Subtilisin a protease composition derived from *Bacillus licheniformis*. Alcalase® is available from Novozymes A/S.

Termamyl SC ®, a *Bacillus* alpha-amylase available from Novozymes A/S.

The following monocomponent endoglucanases and xylanases were applied:

Endoglucanases;			
AA EG I	*Aspergillus aculeatus*		SEQ ID NO:1
AA EG II	*Aspergillus aculeatus*	Cel12b	SEQ ID NO:2
AA EG III	*Aspergillus aculeatus*	Cel12a	SEQ ID NO:16
HI EG I	*Humicola insolens*	Cel12a, GH12	SEQ ID NO:3.
HI EG III	*Humicola insolens*	Cel12a, GH12	SEQ ID NO:4.
HI EG IV	*Humicola insolens*	Cel5a, GH12	SEQ ID NO:5
HI EG V	*Humicola insolens*	Cel45a, GH45	SEQ ID NO:8
TA EG BG025	*Thermoascus aurantiacus*		SEQ ID NO:6
Xylanases			
AA XYL I	*Aspergillus aculeatus*	GH10, Type A	SEQ ID NO:8
AA XYL II	*Aspergillus aculeatus*	GH10, Type A	SEQ ID NO:9
AA XYL III	*Aspergillus aculeatus*	GH11, Type B	SEQ ID NO:10
BH XYL A	*Bacillus halodurans*	GH10, Type A	SWISS PROT P07528.
HI XYL I	*Humicola insolens*	GH11, Type B	SEQ ID NO:11
HI XYL III	Humicola insolens	GH10, Type A	SEQ ID NO:12
MT XYL I	*Myceliophotora thermophila*	GH10, Type A	SEQ ID NO:13
MT XYL III	*Myceliophotora thermophila*	GH11, Type B	SEQ ID NO:14
TL XYL	*Thermomyces lanuginosus*	GH11, Type B	SEQ ID NO:15

Methods

Mash preparation

Unless otherwise stated mashing was performed as follows. Except when noted (e.g. with regard to enzyme dosage) the mash was prepared according to EBC: 4.5.1 using malt grounded according to EBC: 1.1. Mashing trials were performed in 500 ml lidded vessels

incubated in water bath with stirring and each containing a mash with 50 g grist and adjusted to a total weight of 300±0.2 g with water preheated to the initial incubation temperature + 1°C. The wort produced was app. 12% Plato.

Mashing temperature profile

Unless otherwise stated mashing was carried out using an initial incubation temperature at 52°C for 30 minutes, followed by an increasing step to 63°C remaining here for 20 min. The profile is continued with an increasing step to 72°C for 30 min, and mashing off at 78°C for 5 min. All step wise temperature gradients are achieved by an increase of 1°C/min. The mash is cooled to 20°C during 15 min, which result in a total incubation period of 2 hours and 11 min.

Additional methods

Methods for analysis of raw products, wort, beer etc. can be found in *Analytica-EBC*, Analysis Committee of EBC, the European Brewing Convention (1998), Verlag Hans Carl Geranke-Fachverlag. For the present invention the methods applied for determination of the following parameters were as indicated below.

Plato: refractometer.

Beta-glucan: EBC: 8.13.2 (High Molecular weight beta-glucan content of wort: Fluorimetric Method).

Turbidity: EBC: 4.7.1

Filterability: Volume of filtrate (ml) determination: According to EBC: 4.5.1 (Extract of Malt: Congress Mash) subsection 8.2. Filterability: Filtration volume is read after 1 hour of filtration through fluted filter paper, 320 mm diameter. Schleicher and Schüll No.597 ½, Machery, Nagel and Co. in funnels, 200 mm diameter, fitted in 500 ml flasks.

Extract recovery: EBC: 4.5.1 (Extract of Malt: Congress Mash, Extract in dry). The term extract recovery in the wort is defined as the sum of soluble substances (glucose, sucrose, maltose, maltotriose, dextrins, protein, gums, inorganic, other substances) extracted from the grist (malt and adjuncts) expressed in percentage based on dry matter. The remaining insoluble part is defined as spent grains.

$$a)\, E_1 = \frac{P(M+800)}{100-P}$$

$$b)\, E_2 = \frac{E_1 \cdot 100}{100-M}$$

where:

E_1 = the extract content of sample, in % (m/m)

E_2 = the extract content of dry grist, in % (m/m)

P = the extract content in wort, in % Plato

M = the moisture content of the grist, in % (m/m)

800 = the amount of destilled water added into the mash to 100 g of grist

Viscosity: Automated Microviscometer (AMVn) is based on the rolling ball principle. The sample to be measured is introduced into a glass capillary in which a steel ball rolls. The viscous properties of the test fluid can be determined by measuring the rolling time of the steel ball. The rolling time t_0 of a ball over a defined measuring distance in a capillary is measured. The dynamic viscosity η of the sample is calculated from the calibration constant $K_1(\alpha)$ of the measuring system, the rolling time t_0 and the difference of density $\Delta\rho$ between the ball and the sample. The following equation is used:

$$\eta = K_1(\alpha) \cdot t_0 \cdot (\rho_k - \rho_s), \text{ where}$$

η = Dynamic viscosity of the sample, $[mPa \cdot s]$

$K(\alpha)$ = Calibration constant for the Measuring system $[mPa \cdot scm^3 / g]$

t_0 = Rolling time for 100mm $[s]$

ρ_k = Ball density $[7{,}85 g / cm^3]$

ρ_s = Densityof the sample measured $[g / cm^3]$

The viscosity is presented based on the extract (Plato°) as is, or converted to 8,6°Plato based upon a Congress mashing procedure.

Example 1. Characterisation of xylanases using binding assay

Production of fibre fractions

Soluble fibre fraction of barley was produced as follows:

1. 50 kg of barley was milled and slurred into 450 kg water at 50°C under stirring.
2. The extraction was carried out for 30 minutes under stirring.
3. Using a preheated decanter centrifuge at 50°C, and a solids ejecting centrifuge a particle free and clarified fraction was prepared.
4. The clarified fraction was ultra filtered at 50°C on a tubular membrane with a cut-off value of 20000 Dalton. The ultra filtration process was continued until the viscosity increased and the flow was reduced significantly in the system.

5. The concentrated fraction was collected and lyophilized.

Insoluble fibre fraction of barley was produced as follows:

1. 50 kg of barley was milled and slurred into 450 kg of water at 50°C. 0.25 kg of Termamyl SC was added and the solution was heated to 85°C under stirring. The reaction was carried out for 30 minutes. A sample was taken for starch analysis by iodine test.
2. The sample was centrifuged for 5 min at 3000 x g (in 10 ml centrifuge vial). °Plato was measured by using a refractometer on the supernatant. Starch conversion was followed by iodine colour reaction; if blue starch was remaining.
3. The reaction was continued until °Plato has stabilized. The reaction product was ready for centrifugation.
4. The centrifugation was carried out using a decanter. The separation was carried out at 75°C, and a clear and particle free supernatant was obtained. This fraction was discarded. Only the solid fraction was used in the following process.
5. The collected solid fraction was slurred into 500 kg of hot water. The temperature of this slurry was adjusted to 50°C.
6. pH was adjusted to 7.5 using NaOH. A hydrolysis reaction was carried out using 125 g Alcalase 2.4 L. During the hydrolysis pH was maintained at pH=7.5 (pH-stat) and the reaction time was 120 minutes. Hereafter the reaction was left stirred without pH-stat at T=50°C over night.
7. pH was then adjusted to 6.5 using HCl.
8. The reaction mixture was centrifuged using the decanter.
9. The solid fraction was collected and washed with 500 L of water at 50°C for 30 minutes. The centrifugation step and washing step was repeated.
10. This washed solid fraction was lyophilized.

<u>Fibre fraction analysis</u>

The sugar composition of the fibre fractions was analysed as follows: 1 g of fibre was added 50 mL of 1 M HCl and incubated at 100 °C for 2 hours with shaking. After this treatment the reaction mixture was immediately cooled on ice and 11 mL of 4 M NaOH was added to neutralise the mixture. The content of arabinose, galactose, glucose and xylose was quantified using a Dionex BioLC system equipped with a CarBoPac PA-1 column as described in Sørensen et al. (2003) Biotech. Bioeng. vol. 81, No. 6, p. 726-731. The results are shown in table 1.

Table 1. Content (g/kg) of the individual sugars in the fibre fractions from barley

	Arabinose	Galactose	Glucose	Xylose
Soluble fibers	34.9	14.8	486.6	38.1
Insoluble fibers	102.3	10.4	42.3	207.2

Xylanases binding assay

The xylanases binding assay was performed as follows: The fibre (10 mg) was washed in an Eppendorf tube by whirly-mixing with 500 microL of acetate buffer (50 mM, pH 5.5, 0.1 % Triton X-100) before being centrifuged for 2 min at 13000 g. Washing and centrifuging was performed twice. The solution containing the enzyme* (500 microL, in acetate buffer pH 5.5) was then added to the substrate and the mixture was thoroughly whirly-mixed and kept in an ice bath for 10 min. The Eppendorf tube containing the reaction mixture was then centrifuged at14000 g for 3 min where after initial and residual activity was determined by using as substrate 0.2% AZCL-Arabinoxylan from wheat (Megazyme) in 0.2 M Na-phosphate buffer pH 6.0 + 0.01% Triton-x-100. A vial with 900 microL substrate was preheated to 37°C in a thermomixer. 100 microL enzyme sample was added followed by incubation for 15 min at 37°C and maximum shaking. The vial was placed on ice for 2 min before being centrifuged for 1 min at 20.000 g. From the supernatant 2 x 200 microL was transferred to a microtiter plate and endpoint OD 590 nm was measured and compared relative to a control. The control was 100 microL enzyme sample incubated with 900 microL 0.2 M Na-phosphate buffer pH 6.0 + 0.01% Triton-x-100 instead of substrate and subsequently all activity is recovered in the supernatant and this value set to 1. The results are shown in table 2.

The two xylanases having the highest soluble/insoluble barley fibre binding ratio, Xylanase II and I from *A. aculeatus*, were also the two xylanases having the best performance in the mashing trials.

Table 2. Soluble/insoluble barley fibre binding ratio. Relative activity measured in the supernatant after 10 min incubation with soluble and insoluble barley fibre fractions and the resulting ratios between activities measured in the supernatants over soluble and insoluble barley fibre.

Xylanase	GH Family	Insoluble barley fibers	Soluble barley fibers	Ratio Soluble/
Aspergillus aculeatus Xyl II	10	86	104	1.21
Aspergillus aculeatus Xyl I	10	105	56	0.52
Humicola insolens Xyl II	11	82	37	0.45
Thermomyces lanuginosus Xyl	11	83	14	0.17
Humicola insolens Xyl I	11	74	7	0.09
Bacillus halodurans Xyl A	11	79	7	0.09

Example 2. Characterization of xylanase specificity

High field Nuclear Magnetic Resonance (^{1}H NMR) was applied to identify differences in xylanase specificity towards insoluble wheat arabinoxylan (AX) (insoluble, Megazyme). In ^{1}H NMR, arabinoxylan or oligosaccharides hereof (AXO) show signals (chemical shifts) around 5.0-5.5 ppm arising from the anomeric protons H-1 from the α-L-arabinofuranoside units. The individual differences among these depending on their local surroundings can be used to evaluate the specificity of xylanases towards this highly branched polymer.

The standard condition was 10 mg/mL of AX in 50 mM acetate buffer, pH 5.5 was incubated with 0.1 XU/mL for 120 min at 30°C. The xylanase was then inactivated (95°C, 20 min) and the solution concentrated on a rotary evaporator. The sample was then evaporated twice from D_2O (1 mL) and finally re-suspended in D_2O (~0.8 mL) before being analyzed. ^{1}H NMR spectra were recorded on a Varian Mercury 400 MHz at 30°C. Data were collected over 100 scans and the HDO signal was used as a reference signal (4.67 ppm).

Degradation of AX with a xylanase changes the ^{1}H NMR spectra according to the specificity of the enzyme. Thus, the chemical shift of the arabinofuranoside H-1 changes if the arabinose in the resulting oligosaccharide is located on a terminal xylose as compared to an "internal" xylose. This will be the result if the xylanase is capable at placing a substituted xylose unit in its +1 subsite. Using the applied conditions it was found that all tested GH10 xylanases was able to do this, whereas no GH11 xylanases having the characteristic were found. Type A refers to a xylanase that cleaves next to branched residues (leaving terminal substituted xylose oligosaccharides) whereas Type B refers to a xylanase that cleaves between unsubstituted xylose units giving internal substituted units only. Type A xylanases are also capable at cleaving between unsubstituted xylose units. Examples of Type A and Type B xylanase identified by the inventors are shown in table 3. For the invention a type A xylanase is preferred.

Table 3. Examples of Type A and Type B xylanases

Type A	Type B
Aspergillus aculeatus Xyl I	Biobake (Quest)
Aspergillus aculeatus Xyl II	*Humicola insolens* Xyl I
Bacillus halodurans Xyl A	*Myceliophotora thermophila* Xyl III
Humicola insolens Xyl III	*Thermomyces lanuginosus* Xyl I
Myceliophotora thermophila Xyl I	

Even within the type A xylanases the preference for cleavage next to branched residues or between unsubstituted xylose varies as shown in table 4, where the ratio $I_{1,3terminal}/I_{1,3internal}$ relates to the ratio between the respective integrals of the two types of protons. Thus, type A cleavage result in an increase of $I_{1,3terminal}$ whereas type B does not. The chemical shifts for the two types of protons are: 1,3-linked arabinofuranoside H-1 on terminal xylose: 5.26 ppm and 1,3-linked arabinofuranoside H-1 on internal xylose: 5.32 ppm. For the invention a type A xylanase having a $I_{1,3terminal}/I_{1,3internal}$ ratio of at least 0.25, such as at least 0.30, al least 0.40, at least 0.50, or even at least 0.60, is preferred.

Table 4. Xylanases specificity, preference for cleavage next to branched residues or between unsubstituted xylose

	$I_{1,3terminal}/I_{1,3internal}$
Myceliophotora thermophila Xyl I	0.64
Aspergillus aculeatus Xyl II	0.60
Humicola insolens Xyl III	0.30
Aspergillus aculeatus Xyl I	0.28

Example 3. Characterization of endoglucanase specificity

Specificity of endoglucanases was studied by analyzing degradation products upon incubation with barley beta-glucan. Eppendorf tubes with 0.1 and 10 microgram/ml purified enzyme and 5 mg/ml barley beta-glucan (Megazyme, low viscosity) in 50 mM sodium acetate, 0.01% Triton X-100 at pH 5.5 were incubated in an Eppendorf thermomixer at 50°C with agitation.

Enzymes tested were *endoglucanase* EG I from *Humicola insolens*, endoglucanase EG III from *Humicola insolens*, endoglucanase *Humicola insolens* EG IV, *Aspergillus aculeatus* EG II (XG5, Cel12B), and *Aspergillus aculeatus* EG III (XG53, Cel12A).

Samples were withdrawn between 1 and 21.5 hours and inactivated by heating for 30 min at 95°C. Half the volume of each sample was degraded with lichenase (0.085 microgram/ml, Megazyme, from Bacillus subtilis) in 50 mM MES, 1 mM CaCl2, pH 6.5 for 2 hours at 50°C, after which the lichenase was inactivated by heating to 95°C for 30 min. Samples with and without lichenase treatment were diluted appropriately with Milli Q water

and analyzed on a Dionex DX-500 HPAEC-PAD system (CarboPac PA-100 column; A buffer: 150 mM NaOH; B buffer: 150 mM NaOH + 0.6 M sodium acetate; Flow rate: 1 ml/min. Elution conditions: 0-3 min: 95% A + 5% B; 3-19 min: linear gradient: 95% A+ 5% B to 50% A and 50% B; 19-21 min: linear gradient: 50% A + 50% B to 100% B; 21-23 min: 100% B). As reference on the Dionex system a mixture of cellooligosaccharides was used (DP1 to DP6, 100 microM of each). Peaks in chromatograms were identified using the cellooligo references and known composition of barley beta-glucan after lichenase treatment (e.g. Izydorczyk, M.S., Macri, L.J., & MacGregor, A.W., 1997, Carbohydrate Polymers, 35, 249-258). Quantification of peaks in chromatograms was done using response factors obtained for cellooligo references and assuming that response factor was identical for oligosaccharides of same DP with beta-1,3 bonds. For oligosaccharides larger than DP6 response factor of DP6 was used.

From the analysis of degradation products with EG I from *Humicola insolens* (Tables 5 and 6), it was found that the enzyme is able to degrade both beta-1,3 and beta-1,4 bonds. Initially, cellobiose, cellotriose and to some extent laminaribiose are the main products increasing after lichenase treatment. This indicates that beta-1,3 bonds are accepted between glucose units in subsites -4/-3, -5/-4 and +1/+2. The main products with highest enzyme dosage (10 microgram/ml) and longest incubation time (21.5 hours) were found to be glucose and cellobiose.

With EG III from *Humicola insolens* (Tables 7 and 8) the main products after 21.5 hours and 10 microgram/ml enzyme were tetraoses (mainly Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu and Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu but not Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu), pentaoses (probably mainly Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu and Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu) and larger oligomers. Composition of degradation products after lichenase treatment shows that the enzyme exclusively degrades the beta-1,4 bonds in beta-glucan. Futhermore, the the beta-1,4 linkages that are hydrolysed are mainly those not hydrolysed by lichenases (without adjacent beta-1,3 bond towards the non-reducing end). That the amount of Glu(beta-1,4)Glu(beta-1,3)Glu ("Lic3") after lichenase treatment does not decrease significantly even after 21.5 hours with 10 microgram/ml indicates that the enzyme only has limited activity on stretches with only two beta-1,4 bonds between beta-1,3 linkages. The appearance of significant amounts of glucose and laminaribiose but not cellobiose or cellotriose after lichenase treatment indicates that beta-1,3 bonds are accepted between glucose units in subsites -3/-2 and +1/+2 but not between -4/-3 or -5/-4.

The enzyme EG IV from *Humicola insolens* mainly degrades the beta-glucan to larger oligomers (Tables 9 and 10), but after 21.5 hours with 10 microgram/ml enzyme substantial amounts of cellobiose and oligomers of DP4 (probably mainly Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu and Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu) are formed. The enzyme degrades about equal amounts of beta-1,4 and beta-1,3 bonds in beta-glucan and

the beta-1,4 bonds cleaved seem to be those without an adjacent beta-1,3 bond towards the non-reducing end (unlike lichenases). Lichenase treatment gives increased cellotriose already after limited hydrolysis with EG IV, whereas cellobiose and glucose only appear after more extensive hydrolysis with EG IV. This indicates that beta-1,3 bonds are better accepted between glucose in subsites -5/-4 than between -4/-3 and especially -3/-2. The appearance of laminaribiose after lichenase treatment shows that beta-1,3 bonds are also accepted between glucose in subsites +1/+2.

With *Aspergillus aculeatus* EGII (XG5, Cel12B), glucose is seen to be the main low molecular weight product (Tables 11 and 12). Lichenase treatment of samples with little degradation of beta-glucan by EG II gives increase of cellobiose, cellotriose and laminaribiose but not glucose. This indicates that beta-1,3 bonds are accepted between glucose units in subsites -5/-4, -4/-3 and +1/+2 but probably not -3/-2. Thus, the glucose liberated by EG II is probably released by exo-action on degradation products. The enzyme is able to hydrolyse both beta-1,4 and beta-1,3 bonds although beta-1,4 linkages seem to be preferred. After 20 hours with the highest enzyme concentration, the beta-glucan is seen to be almost totally degraded to glucose.

The *Aspergillus aculeatus* EG III (XG53, Cel12A) rapidly degrades the beta-glucan giving oligomers of DP4 (mainly Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu and Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu) and DP5 (mainly Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu but also some Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu and Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu) (Tables 13 and 14). After 20 hours with the highest enzyme concentration significant amounts of cellobiose, glucose and cellotriose are also formed. Lichenase treatment of samples gives increase of glucose, cellotriose and laminaribiose and especially cellobiose. This indicates that beta-1,3 bonds may be preferred between glucose units in subsites -4/-3 but are also accepted between -5/-4, -3/-2 and +1/+2. The enzyme is capable of degrading both beta-1,4 and beta-1,3 linkages.

Table 5: Degradation products of barley beta-glucan with endoglucanase *Humicola insolens* EG I given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.10	0.28	0.35	3.68	15.45	40.96
Cel_2	0.40	0.93	1.61	13.80	27.32	28.76
Cel_3	0.69	1.64	2.38	9.91	6.00	0.00
Cel_4	0.25	0.48	0.68	2.37	0.85	0.00
Cel_5	0.00	0.40	0.35	1.66	0.11	0.00
Cel_6	0.00	0.00	0.00	0.00	0.00	0.00
Lam_2	0.00	0.00	0.13	0.07	0.06	2.12
DP_3	0.63	0.00	0.06	0.86	3.51	5.33
DP_4	0.00	0.00	0.08	1.29	4.40	4.13
DP_5	0.87	2.38	3.65	22.91	23.44	9.14
DP_6	1.12	2.66	4.50	16.08	4.16	3.41
DP>6	95.93	91.23	86.30	27.38	14.70	6.16

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 6. Degradation products of barley beta-glucan with endoglucanase *Humicola insolens* EG I and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.14	0.17	0.45	5.15	16.24	43.66
Cel_2	3.38	4.99	10.09	36.73	43.18	35.48
Cel_3	1.24	3.31	5.26	14.14	6.42	0.17
Cel_4	0.21	0.79	1.39	3.79	0.90	0.00
Cel_5	0.00	0.16	0.62	1.18	0.84	0.00
Cel_6	0.00	0.00	0.00	0.00	0.00	0.00
Lam_2	2.95	2.43	3.58	9.90	7.53	4.99
DP_3	61.59	58.54	52.42	3.56	6.49	5.46
DP_4	20.92	19.72	16.97	5.19	6.01	4.58
DP_5	4.33	4.10	5.46	13.49	9.44	2.84
DP_6	2.23	2.88	2.75	4.84	1.00	2.47
DP>6	3.01	2.91	0.29	2.04	1.96	0.34

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 7: Results upon degradation of barley beta-glucan with endoglucanase *Humicola insolens* EG III given as weight % of degradation products.						
Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.00	0.08	0.06	0.30	0.25	0.68
Cel2	0.00	0.00	0.00	0.08	0.01	0.53
Cel3	0.00	0.00	0.00	0.00	0.00	0.00
Cel4	0.00	0.00	0.00	0.00	0.03	0.00
Cel5	0.00	0.00	0.00	0.00	0.00	0.00
Cel6	0.00	0.00	0.00	0.00	0.00	0.00
Lam2	0.00	0.00	0.00	0.00	0.03	0.24
DP3	1.07	0.00	0.00	0.66	0.05	0.00
DP4	0.00	0.14	0.53	5.99	7.95	39.00
DP5	0.78	0.00	0.76	4.50	6.68	25.08
DP6	0.00	0.00	0.36	1.26	1.92	7.08
DP>6	98.15	99.78	98.29	87.22	83.09	27.31
Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.						

Table 8: Results upon degradation of barley beta-glucan with endoglucanase *Humicola insolens* EG III and subsequent lichenase degradation given as weight % of degradation products.						
Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.15	0.29	1.37	6.70	14.43	13.80
Cel2	0.32	0.19	0.44	0.21	0.24	0.90
Cel3	1.03	0.00	2.01	0.93	0.45	0.15
Cel4	0.00	0.00	0.00	0.00	0.03	0.00
Cel5	0.80	0.00	0.00	0.00	0.00	0.00
Cel6	0.00	0.00	0.00	0.00	0.00	0.00
Lam2	4.10	1.26	2.10	7.43	13.20	13.77
"Lic3"	59.81	65.09	61.00	47.76	40.48	56.20
"Lic4"	22.63	23.12	21.54	18.78	7.08	9.72
"Lic5"	4.24	4.45	4.92	10.59	15.64	2.88
"Lic6"	3.91	2.82	3.67	3.28	2.90	2.05
"Lic7"	3.00	2.79	2.93	4.32	5.54	0.55
Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.						

Table 9: Degradation products of barley beta-glucan with endoglucanase *Humicola insolens* EG IV given as weight % of degradation products. Glu: Glucose.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.00	0.00	0.00	0.13	0.63	0.66
Cel_2	0.14	0.38	1.07	7.02	5.51	12.11
Cel_3	0.09	0.19	0.77	2.89	1.72	1.02
Cel_4	0.81	0.20	0.34	1.10	0.55	0.13
Cel_5	0.15	0.28	0.30	0.00	0.00	0.00
Cel_6	0.00	0.29	0.00	0.00	0.00	0.00
Lam_2	0.00	0.00	0.00	0.00	0.04	0.16
DP_3	0.00	0.00	0.00	0.68	0.00	0.11
DP_4	0.00	0.00	0.00	1.03	1.83	12.77
DP_5	0.18	0.21	0.07	0.59	0.71	3.25
DP_6	0.00	0.13	0.26	5.78	6.04	2.44
DP>6	98.63	98.32	97.20	80.77	82.96	67.36

Celi: Cellooligo of DP i. Lam2: Laminaribiose. DPi: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 10: Degradation products of barley beta-glucan with endoglucanase *Humicola insolens* EG IV and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.07	0.05	0.09	1.83	2.93	7.24
Cel_2	0.40	0.50	1.97	4.53	7.23	19.84
Cel_3	1.45	2.05	5.59	11.84	12.00	6.94
Cel_4	0.81	1.13	1.90	1.48	0.57	0.08
Cel_5	0.00	0.00	0.00	0.00	0.00	0.00
Cel_6	0.00	0.00	0.00	0.00	0.00	0.00
Lam_2	2.10	3.92	3.82	5.43	7.41	11.87
DP_3	63.45	61.92	60.26	54.03	47.65	30.59
DP_4	22.95	23.32	21.96	16.11	11.03	15.88
DP_5	4.97	4.89	3.46	3.03	2.51	4.01
DP_6	3.82	0.20	0.49	0.11	3.04	1.00
DP>6	0.00	1.93	0.47	1.60	5.62	2.55

Celi: Cellooligo of DP i. Lam2: Laminaribiose. DPi: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 11: Degradation products of barley beta-glucan with endoglucanase *Aspergillus aculeatus* EG II (XG5, Cel12B) given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.16	0.16	16	16
Incubation time (hours)	1	20	1	20
Glu	0.17	2.30	33.64	99.25
Cel_2	0.00	0.00	0.54	0.00
Cel_3	0.00	0.00	0.80	0.00
Cel_4	0.00	0.00	0.52	0.00
Cel_5	0.00	0.00	0.11	0.00
Cel_6	0.00	0.00	0.00	0.00
Lam_2	0.00	0.12	2.97	0.00
DP_3	0.00	0.45	0.09	0.16
DP_4	0.00	0.06	1.85	0.02
DP_5	0.00	0.20	1.69	0.16
DP_6	0.00	0.28	4.58	0.00
DP>6	99.83	96.59	53.42	0.41

Glu: Glucose. Celi: Cellooligo of DP i. Lam2: Laminaribiose. DPi: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 12: Degradation products of barley beta-glucan with endoglucanase *Aspergillus aculeatus* EG II (XG5, Cel12B) and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.016	0.016	1.6	1.6
Incubation time (hours)	1	20	1	20
Glu	0.20	2.21	26.22	99.53
Cel_2	4.88	0.61	1.31	0.00
Cel_3	3.76	3.44	4.10	0.00
Cel_4	0.00	0.20	0.82	0.00
Cel_5	0.00	0.88	0.00	0.00
Cel_6	0.00	0.00	0.00	0.00
Lam_2	0.17	2.17	9.95	0.00
DP_3	61.15	59.72	36.11	0.27
DP_4	23.43	21.49	14.35	0.04
DP_5	3.62	3.83	3.38	0.16
DP_6	0.08	2.52	2.20	0.00
DP>6	2.51	2.94	1.55	0.00

Glu: Glucose. Celi: Cellooligo of DP i. Lam2: Laminaribiose. DPi: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 13: Degradation products of barley beta-glucan with endoglucanase *Aspergillus aculeatus* EG III (XG53, Cel12A) given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	10	10
Incubation time (hours)	1	20	1	20
Glu	0.05	0.23	1.42	13.28
Cel_2	0.09	0.78	4.20	20.68
Cel_3	0.15	1.21	2.69	7.57
Cel_4	0.17	0.91	1.19	0.00
Cel_5	0.08	0.00	0.00	0.00
Cel_6	0.00	0.00	0.00	0.00
Lam_2	0.00	0.15	0.25	0.03
DP_3	0.33	0.16	0.00	0.42
DP_4	0.26	8.71	40.77	33.42
DP_5	1.24	15.49	30.18	20.94
DP_6	0.79	8.69	0.26	1.66
DP>6	96.83	65.67	19.04	2.01

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 14: Degradation products of barley beta-glucan with endoglucanase *Aspergillus aculeatus* EG III (XG53, Cel12A) and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	10	10
Incubation time (hours)	1	20	1	20
Glu	1.08	6.84	7.12	16.22
Cel_2	3.37	16.31	21.82	30.46
Cel_3	3.90	5.25	4.40	7.66
Cel_4	0.70	2.35	0.65	0.03
Cel_5	0.58	0.00	0.00	0.10
Cel_6	0.00	1.22	0.00	0.00
Lam_2	4.12	16.93	16.24	5.07
DP_3	57.22	33.12	6.11	0.99
DP_4	18.69	12.16	38.91	35.81
DP_5	4.39	1.46	2.41	2.62
DP_6	3.44	2.16	0.26	0.78
DP>6	2.51	2.19	2.08	0.26

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Example 4. Mashing and filtration performance

A conventional standard treatment of Ultraflo® 2.7 mg EP/kg dry matter (dm) grist (index 1,000) was compared to an experimental treatment with Ultraflo® 1.4 mg EP/kg dm grist supplemented with various endoglucanases. A dosage of 0.2 g Ultraflo® /kg DM grist equals 2.7 mg enzyme protein/kg dm grist.

Table 15. Effect of *Humicola insolens* EG I endoglucanase (Cel 7b, GH 7) and *Humicola insolens* EG V endoglucanase, (Cel 45a, GH45).					
	Beta-glucan	Extract	Viscosity	Filterability	Best Performing
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	-
Ultraflo® 1.4 mg EP/kg dm + HI EG I 1,25 mg EP/kg dm	1.184	0.997	1.032	0.904	-
Ultraflo® 1.4 mg EP/kg dm + HI EG V 1,25 mg EP/kg dm	2.986	0.996	1.033	0.865	-
Ultraflo® 1.4 mg EP/kg dm + HI EG I 8 mg EP/kg dm	0.377	0.992	1.021	0.962	** beta -glucan
Ultraflo® 1.4 mg EP/kg dm + HI EG V 8 mg EP/kg dm	3.262	1.000	1.055	0.865	-
Beta-glucan (n=4), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min					

Ultraflo® 1.4 mg EP/kg dm supplemented with *H. insolens* EG I, Cel 7b (GH 7) 8 mg EP/kg dm reduced beta-glucan compared to the standard treatment (index 1.000).

Table 16. Effect of *Humicola insolens* EGIII endoglucanase, (Cel 12a, GH12) and *Humicola insolens* EG IV endoglucanase, (Cel 5a, GH12).						
	Beta-glucan	OD	Extract	Viscosity	Filterability	Best Performing
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	1.000	-
Ultraflo® 1.4 mg EP/kg dm + HI EG IV 1,25 mg EP/kg dm	3.019	0.975	1.002	1.002	0.979	-
Ultraflo® 1.4 mg EP/kg dm + HI EG III 1,25 mg EP/kg dm	0.628	0.949	1.000	0.999	0.957	-
Ultraflo® 1.4 mg EP/kg dm + HI EG IV 8,0 mg EP/kg dm	2.045	1.013	0.999	1.006	1.085	-
Ultraflo® 1.4 mg EP/kg dm + HI EG III 8,0 mg EP/kg dm	0.341	0.937	1.003	0.938	1.085	*** beta-glucan, viscosity, filterability
Beta-glucan (n=4), OD (n=2), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min						

The *H. insolens*, endoglucanase III, (Cel 12a, GH12) and Ultraflo® 1.4 mg EP/kg dm reduced the beta-glucan, O.D and viscosity while also improving filterability compared to the standard treatment.

Table 17. Effect of *Thermoascus aurantiacus* endoglucanase (GH 5).						
	Beta-glucan	**OD**	**Extract**	**Viscosity**	**Filterability**	**Best Performing**
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	1.000	
Ultraflo® 1.4 mg EP/kg dm + AT EG 5 1,25 mg EP/kg dm	1.627	1.065	1.002	1.015	1.037	
Ultraflo® 1.4 mg EP/kg dm + AT EG 8 mg EP/kg dm	0.432	1.033	1.001	1.017	1.000	** beta -glucan
Beta-glucan (n=4), OD (n=2), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8.6° Plato, cP), Filterability (n=2) after 10 min						

Ultraflo® 1.4 mg EP/kg dm supplemented with the *T. aurantiacus* endoglucanase BG025 (GH 5) reduced the beta-glucan level significantly compared to the standard treatment.

A conventional standard treatment of Ultraflo® 0.2 g/kg DM grist (index 1,000) was compared to an experimental treatment with Ultraflo® 0.1 g/kg DM grist supplemented with various xylanases.

None of the two GH 11, type B xylanases from the fungi *Bh* and *Cc* had any positive effect on beta-glucan, OD, Extract recovery, viscosity or filterability.

Table 18. Effect of *Bh* xylanase B (GH 11, type B) & *Cc* xylanase II (GH 11 type B).						
	Beta-glucan	**OD**	**Extract**	**Viscosity**	**Filterability**	**Best Performing**
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	1,000	-
Ultraflo® 1.4 mg EP/kg dm + BH XYL 0,7 mg EP/kg dm	3.223	1.100	1.000	1.032	1,082	-
Ultraflo® 1.4 mg EP/kg dm + CC XYL II 0,7 mg EP/kg dm	3.279	1.025	0.998	1.028	1,012	-
Ultraflo® 1.4 mg EP/kg dm + BH XYL B 5 mg EP/kg dm	3.231	1.025	1.000	1.048	1,035	-
Ultraflo® 1.4 mg EP/kg dm + CC XYL II 5 mg EP/kg dm	3.213	1.038	1.003	1.030	1,082	-
Beta-glucan (n=4), OD (n=2), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min						

Table 19. Effect of *Aspergillus aculeatus* xylanase I (GH10, type A) and *Mycellophotora thermophila* xylanase III (GH 11, type B).

	Beta-glucan	OD	Extract	Viscosity	Filterability	Best Performing
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	1,000	-
Ultraflo® 1.4 mg EP/kg dm+ AA XYL I 5 mg EP/kg dm	3.401	1.125	1.006	0.981	1,143	*** Viscosity, filterability
Ultraflo® 1.4 mg EP/kg dm+ MT XYL II 5 mg EP/kg dm	3.740	0.990	1.005	1.019	0,939	-
Ultraflo® 1.4 mg EP/kg dm+ AA XYL I 0,7 mg EP/kg dm	3.894	1.010	1.006	1.006	0,980	-
Ultraflo® 1.4 mg EP/kg dm+ MT XYL II 0,7 mg EP/kg dm	3.218	0.927	1.007	1.020	0,776	-

Beta-glucan (n=4), OD (n=2), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

Table 20. Effect of *Thermomyces lanuginosus* xylanase (GH 11, type B) and *Aspergillus aculeatus* xylanase II (GH10, type A).

	Beta-glucan	Extract	Viscosity	Filterability	Best Performing
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	-
Ultraflo® 1.4 mg EP/kg dm + AA XYL II 0,7 mg EP/kg dm	2.296	1.002	0.965	0.981	-
Ultraflo® 1.4 mg EP/kg dm + TL XYL 0,7 mg EP/kg dm	2.375	0.994	1.015	0.868	-
Ultraflo® 1.4 mg EP/kg dm + AA XYL II 5 mg EP/kg dm	2.152	1.000	0.970	1.075	*** Viscosity, filterability
Ultraflo® 1.4 mg EP/kg dm + TL XYL 5 mg EP/kg dm	2.357	1.004	1.032	0.906	-

Beta-glucan (n=4), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

The *Aspergillus aculeatus* xylanase I and *Aspergillus aculeatus* xylanase II reduced viscosity as well as improved filterability compared to the standard treatment.

A conventional standard treatment of Ultraflo® 0.2 g/kg dm grist (index 1.000) was compared to an experimental treatment with Viscozyme 0.1 g or 0 g/kg dm grist supplemented with *Aspergillus aculeatus* xylanase II and various endoglucanases.

Table 21. Effect of *Aspergillus aculeatus* betaglucanase EGI (XG 5) and *Aspergillus aculeatus* endoglucanase EGIII (XG53) in combination with *Aspergillus aculeatus* xylanase II and/or the Viscozyme® endoglucanase composition.					
	Beta-glucan	Extract	Viscosity	Filterability	Best Performing
Ultraflo®: 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	
AA EG II 4 mg EP/kg dm AA XYL II 4 mg EP/kg dm Viscozyme® 1.7 mg EP/kg dm	5.795	0.997	0.981	1.140	
AA EG III 1 mg EP/kg dm AA XYL II 4 mg EP/kg dm Viscozyme® 1.7 mg EP/kg dm	2.631	1.000	0.964	1.118	** viscosity, filterability
AA EG III 2 mg EP/kg dm AA XYL II 4 mg EP/kg dm Viscozyme® 1.7 mg EP/kg dm	1.317	1.003	0.955	1.011	
AA EG III 4 mg EP/kg dm, AA XYL II 4 mg EP/kg dm	0.918	1.004	0.956	1.236	*** beta -glucan, viscosity, filterability
AA EG II 8 mg EP/kg dm AA XYL II 4 mg EP/kg dm	6.601	1.003	0.977	1.096	
Beta-glucan (n=4), Extract % (n=4, based on dry malt), Viscosity (n=4, conv. 8.6° Plato, cP), Filterability (n=2) after 10 min					

A combination of *Aspergillus aculeatus* Xylanase II and *Aspergillus aculeatus* endoglucanase EG III had a significant effect on beta-glucan, viscosity and filterability.

Table 22. Comparison of increasing amounts of Viscozyme® and of a composition of the present invention. Absolute values.				
	Beta-glucan	OD	Extract %	Viscosity
Viscozyme® 3.6 mg EP/kg dm	189	0.030	85.0	1.38
Viscozyme® 9 mg EP/kg dm	155	0.030	85.0	1.35
Viscozyme® 13.5 mg EP/kg dm	127	0.031	85.2	1.34
Viscozyme® 18 mg EP/kg dm	101	0.028	85.5	1.32
Viscozyme® 27 mg EP/kg dm	75	0.030	85.7	1.30
Viscozyme® 3.6 mg EP/kg dm AA EG III 2 mg EP/kg dm AA XYL II 4 mg EP/kg dm	0	0.030	85.8	1.22
Beta-glucan (mg/l, n=4), OD (n=2), Extract % (n=4, extract in dry malt, % (m/m)), Viscosity (n=4, conv. 8.6° Plato, cP).				

A composition comprising Viscozyme® 3.6 mg EP/kg dm, *Aspergillus aculeatus* EG III 2 mg EP/kg dm, and *Aspergillus aculeatus* Xylanase II 4 mg EP/kg dm had a significantly more positive effect on beta-glucan, OD, extract recovery, and viscosity than had a dosage of 7.5 times the conventional standard dosage of Viscozyme® (Std. dosage = 3.6 mg EP/kg dm) (Table 22).

Example 5. Quantification of protein bands in SDS-PAGE gels.

The enzyme composition was diluted 250 times in deionized water and loaded onto a 4-20% Tris-glycine SDS-PAGE gel (Nu Page, Invitrogen) and the electrophoresis was

conducted as described by the manufacturer.

After electrophoresis the gel was stained with GelCode Blue (Pierce) o/n and subsequently decolorized in water to the background became clear.

The resulting gel was then scanned using a densitometer and analyzed by the ImageMaster™ v. 1 0 software from Amersham Biosciences following the protocol from the manufacturer. The results are expressed as %band density of total density in a given lane.

The total amount of protein in the enzyme samples were measured using the Micro BCA kit from Pierce using the protocol supplied with the kit.

CLAIMS

2. A process for production of a mash having enhanced filterability and/or improved extract yield after filtration, which comprises; preparing a mash in the presence of enzyme activities and filtering the mash to obtain a wort, wherein the enzyme activities comprise; a xylanase of GH family 10 present in an amount of at least 15% w/w of the total xylanase and endoglucanase enzyme protein of said composition.

3. The process of the proceeding claim wherein endoglucanase is present, said endoglucanase belonging to a GH family selected from the list consisting of; GH12, GH7 and GH5.

4. The process of the proceeding claims wherein the endoglucanase activity belonging to GH family GH12, GH7 and/or GH5 is present in an amount of at least 40% w/w of the total xylanase and endoglucanase enzyme protein of said composition.

5. The process of the proceeding claims wherein the xylanase of GH family 10 is present in an amount of at least 20%, preferably 25%, such as at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, or even at least 70% w/w of the total xylanase and endoglucanase enzyme protein

6. The process of the proceeding claims wherein the endoglucanase of GH Family 12, 7 and/or 5 endoglucanase is present in an amount of at least 45%, preferably 50%, such as at least 55%, at least 60%, at least 70% or even at least 80% w/w of the total xylanase and endoglucanase enzyme protein.

7. The process of the proceeding claims wherein the xylanase is a type A xylanase.

8. The process of the proceeding claims wherein the xylanase is a type A xylanase having a $I_{1,3terminal}/I_{1,3internal}$ ratio of at least 0.25, such as at least 0.30, al least 0.40, at least 0.50, or even at least 0.60.

9. The process of the proceeding claims wherein the xylanase has a CBM, preferably a CBM of family 1.

10. The process of the proceeding claims wherein the xylanase is a xylanase which in the xylanase binding assay described herein has a barley soluble/insoluble fibre binding ratio of at least 0.50, preferably at least 0.60, more preferably at least 0.70, such as 0.80, 0.90, 1.00, 1.10 or even at least 1.20.

11. The process of the proceeding claims wherein the xylanase is derived from a filamentous fungi such as from a strain of an *Aspergillus* sp., preferably from *Aspergillus aculeatus* (SEQ ID NO:8 or SEQ ID NO:9), from a strain of a *Myceliophotora* sp., preferably from a *Myceliophotora thermophilia* (SEQ ID NO:13), or from a strain of a *Humicola* sp., preferably from *Humicola Insolens* (SEQ ID NO:12).

12. The process of the proceeding claims wherein the xylanase is derived from a bacterium such as from a strain of a *Bacillus*, preferably from *Bacillus halodurans*.

13. The process of the proceeding claims wherein the endoglucanase is an endoglucanase derived from *Humicola* sp., such as the endoglucanase from *Humicola insolens* (SEQ ID NO:3), or the endoglucanase from *H. insolens* (SEQ ID NO:4), from *Thermoascus* sp., such as the endoglucanase derived from *Thermoascus aurantiacus* (SEQ ID NO:6) or from *Aspergillus* sp., such as the endoglucanase derived from *Aspergillus aculeatus* (SEQ ID NO:16).

14. The process of the proceeding claims wherein at least one additional enzyme is present, which enzyme is selected from the list comprising; arabinofuranosidase, ferulic acid esterase and xylan acetyl esterase.

15. A process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising:

   a. testing at least one xylanolytic enzyme for its hydrolytic activity towards insoluble wheat arabinoxylan,

   b. selecting a xylanolytic enzyme which cleaves next to branched residues thereby leaving terminal substituted xylose oligosaccharides.

   c. adding the selected xylanolytic enzyme to the aqueous solution comprising a

starch hydrolysate.

16. A process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising:

   d. testing at least one endoglucanolytic enzyme for its hydrolytic activity towards barley beta-glucan,

   e. selecting a endoglucanolytic enzyme which under the conditions: 10 microgram/ml purified enzyme and 5 mg/ml barley beta-glucan in 50 mM sodium acetate, 0.01% Triton X-100, at pH 5.5 and 50°C, within 1 hour degrades more than 70% of the barley beta-glucan to DP 6 or DP<6,

   f. adding the selected endoglucanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

17. The process of the proceeding claims, wherein the aqueous solution comprising a starch hydrolysate is a mash for beer making or a feed composition

18. A composition comprising;

   g. a GH10 xylanase present in an amount of at least 15% w/w of the total enzyme protein; and/or,

   h. a GH12, GH7 and/or GH5 endoglucanase present in an amount of at least 20% w/w of the total enzyme protein.

19. The composition according to the proceeding claim wherein the xylanase is a type A xylanase, and preferably a type A xylanase having a $I_{1,3terminal}/I_{1,3internal}$ ratio of at least 0.25, such as at least 0.30, al least 0.40, at least 0.50, or even at least 0.60.

20. The composition according to the proceeding claims wherein the xylanase is derived from a filamentous fungi such as from a strain of an *Aspergillus* sp., preferably from *Aspergillus aculeatus* (SEQ ID NO:8 or SEQ ID NO:9), from a strain of a *Myceliophotora* sp., preferably from a *Myceliophotora thermophilia* (SEQ ID NO:13), from a strain of a *Humicola* sp., preferably from *Humicola insolens* (SEQ ID NO:12).

21. The composition according to the proceeding claims wherein the xylanase is derived from a bacterium such as from a strain of a Bacillus, preferably from *Bacillus halodurans*.

22. The composition according to the proceeding claims wherein the endoglucanase is endoglucanase derived from *Humicola* sp., such as the endoglucanase from *Humicola insolens* (SEQ ID NO:3), or the endoglucanase from *H. insolens* (SEQ ID NO:4), from *Thermoascus* sp., such as the endoglucanase derived from *Thermoascus aurantiacus* (SEQ ID NO:6), or from *Aspergillus* sp., such as the endoglucanase derived from *Aspergillus aculeatus* (SEQ ID NO:16).

23. The composition according to the proceeding claims wherein the xylanase of GH family 10 is present in an amount of at least 20%, preferably 25%, such as at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, or even at least 70% w/w of the total xylanase and endoglucanase enzyme protein

24. The composition according to the proceeding claims wherein the endoglucanase of GH Family 12, 7 and/or 5 endoglucanase is present in an amount of at least 45%, preferably 50%, such as at least 55%, at least 60%, at least 70% or even at least 80% w/w of the total xylanase and endoglucanase enzyme protein.

25. Use of a composition according to the proceeding claims in a process comprising reducing the viscosity of an aqueous solution comprising a starch hydrolysate.

26. Use of a composition according to the proceeding claims in a process comprising filtering of an aqueous solution comprising a starch hydrolysate.

27. Use of a composition according to the proceeding claims in a process wherein the aqueous solution comprising a starch hydrolysate is a mash for beer making.

28. Use of a composition according to the proceeding claims in a process wherein the aqueous solution comprising a starch hydrolysate is a feed composition.

ABSTRACT

The present invention relates to a mashing and filtration step in a brewing process and to a composition useful in the mashing and filtration step of a brewing process.

10429-000.ST25

SEQUENCE LISTING

<110> Novozymes A/S

<120> 10429, Mashing Process and Composition

<130> 10429.000-DK

<160> 16

<170> PatentIn version 3.2

<210> 1
<211> 332
<212> PRT
<213> Aspergillus aculeatus

<400> 1

Met Lys Leu Leu Asn Leu Leu Val Ala Ala Ala Ala Ala Gly Ser Ala
1 5 10 15

Val Ala Ala Pro Thr His Glu His Thr Lys Arg Ala Ser Val Phe Glu
20 25 30

Trp Ile Gly Ser Asn Glu Ser Asp Ala Glu Phe Gly Thr Ala Ile Pro
35 40 45

Gly Thr Trp Gly Ile Asp Tyr Ile Phe Pro Asp Thr Ser Ala Ile Ala
50 55 60

Thr Leu Val Ser Lys Gly Met Asn Ile Phe Arg Val Gln Phe Met Met
65 70 75 80

Glu Arg Leu Val Pro Asn Ser Met Thr Gly Ser Tyr Asp Asp Ala Tyr
85 90 95

Leu Asn Asn Leu Thr Thr Val Val Asn Ala Ile Ala Ala Ala Gly Val
100 105 110

His Ala Ile Val Asp Pro His Asn Tyr Gly Arg Tyr Asn Asn Glu Ile
115 120 125

Ile Ser Ser Thr Ala Asp Phe Gln Thr Phe Trp Gln Asn Leu Ala Gly
130 135 140

Gln Phe Lys Asp Asn Asp Leu Val Ile Phe Asp Thr Asn Asn Glu Tyr
145 150 155 160

Asn Thr Met Asp Gln Thr Leu Val Leu Asp Leu Asn Gln Ala Ala Ile
165 170 175

Asp Gly Ile Arg Ala Ala Gly Ala Thr Ser Gln Tyr Ile Phe Ala Glu
180 185 190

Gly Asn Ser Trp Ser Gly Ala Trp Thr Trp Ala Asp Ile Asn Asp Asn

195 200 205

Met Lys Ala Leu Thr Asp Pro Gln Asp Lys Leu Val Tyr Glu Met His
210 215 220

Gln Tyr Leu Asp Ser Asp Gly Ser Gly Thr Ser Gly Val Cys Val Ser
225 230 235 240

Glu Thr Ile Gly Ala Glu Arg Leu Gln Ala Ala Thr Gln Trp Leu Lys
245 250 255

Asp Asn Gly Lys Val Asp Ile Leu Gly Glu Tyr Ala Gly Gly Ala Asn
260 265 270

Asp Val Cys Arg Thr Ala Ile Ala Gly Met Leu Glu Tyr Met Ala Asn
275 280 285

Asn Thr Asp Val Trp Lys Gly Ala Val Trp Trp Thr Ala Gly Pro Trp
290 295 300

Trp Ala Asp Tyr Met Phe Ser Met Glu Pro Pro Ser Gly Pro Ala Tyr
305 310 315 320

Ser Gly Met Leu Asp Val Leu Glu Pro Tyr Leu Gly
325 330

<210> 2
<211> 238
<212> PRT
<213> Aspergillus aculeatus

<400> 2

Met Lys Leu Ser Leu Leu Ser Leu Ala Thr Leu Ala Ser Ala Ala Ser
1 5 10 15

Leu Gln Arg Arg Ser Asp Phe Cys Gly Gln Trp Asp Thr Ala Thr Ala
20 25 30

Gly Asp Phe Thr Leu Tyr Asn Asp Leu Trp Gly Glu Ser Ala Gly Thr
35 40 45

Gly Ser Gln Cys Thr Gly Val Asp Ser Tyr Ser Gly Asp Thr Ile Ala
50 55 60

Trp His Thr Ser Trp Ser Trp Ser Gly Gly Ser Ser Ser Val Lys Ser
65 70 75 80

Tyr Val Asn Ala Ala Leu Thr Phe Thr Pro Thr Gln Leu Asn Cys Ile
85 90 95

Ser Ser Ile Pro Thr Thr Trp Lys Trp Ser Tyr Ser Gly Ser Ser Ile
100 105 110

Val Ala Asp Val Ala Tyr Asp Thr Phe Leu Ala Glu Thr Ala Ser Gly
115 120 125

Ser Ser Lys Tyr Glu Ile Met Val Trp Leu Ala Ala Leu Gly Gly Ala
130 135 140

Gly Pro Ile Ser Ser Thr Gly Ser Thr Ile Ala Thr Pro Thr Ile Ala
145 150 155 160

Gly Val Asn Trp Lys Leu Tyr Ser Gly Pro Asn Gly Asp Thr Thr Val
165 170 175

Tyr Ser Phe Val Ala Asp Ser Thr Thr Glu Ser Phe Ser Gly Asp Leu
180 185 190

Asn Asp Phe Phe Thr Tyr Leu Val Asp Asn Glu Gly Val Ser Asp Glu
195 200 205

Leu Tyr Leu Thr Thr Leu Glu Ala Gly Thr Glu Pro Phe Thr Gly Ser
210 215 220

Asn Ala Lys Leu Thr Val Ser Glu Tyr Ser Ile Ser Ile Glu
225 230 235

<210> 3
<211> 435
<212> PRT
<213> Humicola insolens

<400> 3

Met Ala Arg Gly Thr Ala Leu Leu Gly Leu Thr Ala Leu Leu Leu Gly
1 5 10 15

Leu Val Asn Gly Gln Lys Pro Gly Glu Thr Lys Glu Val His Pro Gln
20 25 30

Leu Thr Thr Phe Arg Cys Thr Lys Arg Gly Gly Cys Lys Pro Ala Thr
35 40 45

Asn Phe Ile Val Leu Asp Ser Leu Ser His Pro Ile His Arg Ala Glu
50 55 60

Gly Leu Gly Pro Gly Gly Cys Gly Asp Trp Gly Asn Pro Pro Pro Lys
65 70 75 80

Asp Val Cys Pro Asp Val Glu Ser Cys Ala Lys Asn Cys Ile Met Glu
85 90 95

Gly Ile Pro Asp Tyr Ser Gln Tyr Gly Val Thr Thr Asn Gly Thr Ser
100 105 110

Leu Arg Leu Gln His Ile Leu Pro Asp Gly Arg Val Pro Ser Pro Arg
115 120 125

Val Tyr Leu Leu Asp Lys Thr Lys Arg Arg Tyr Glu Met Leu His Leu
130 135 140

Thr Gly Phe Glu Phe Thr Phe Asp Val Asp Ala Thr Lys Leu Pro Cys
145 150 155 160

Gly Met Asn Ser Ala Leu Tyr Leu Ser Glu Met His Pro Thr Gly Ala
165 170 175

Lys Ser Lys Tyr Asn Pro Gly Gly Ala Tyr Tyr Gly Thr Gly Tyr Cys
180 185 190

Asp Ala Gln Cys Phe Val Thr Pro Phe Ile Asn Gly Leu Gly Asn Ile
195 200 205

Glu Gly Lys Gly Ser Cys Cys Asn Glu Met Asp Ile Trp Glu Ala Asn
210 215 220

Ser Arg Ala Ser His Val Ala Pro His Thr Cys Asn Lys Lys Gly Leu
225 230 235 240

Tyr Leu Cys Glu Gly Glu Glu Cys Ala Phe Glu Gly Val Cys Asp Lys
245 250 255

Asn Gly Cys Gly Trp Asn Asn Tyr Arg Val Asn Val Thr Asp Tyr Tyr
260 265 270

Gly Arg Gly Glu Glu Phe Lys Val Asn Thr Leu Lys Pro Phe Thr Val
275 280 285

Val Thr Gln Phe Leu Ala Asn Arg Arg Gly Lys Leu Glu Lys Ile His
290 295 300

Arg Phe Tyr Val Gln Asp Gly Lys Val Ile Glu Ser Phe Tyr Thr Asn
305 310 315 320

Lys Glu Gly Val Pro Tyr Thr Asn Met Ile Asp Asp Glu Phe Cys Glu
325 330 335

Ala Thr Gly Ser Arg Lys Tyr Met Glu Leu Gly Ala Thr Gln Gly Met
340 345 350

Gly Glu Ala Leu Thr Arg Gly Met Val Leu Ala Met Ser Ile Trp Trp
355 360 365

Asp Gln Gly Gly Asn Met Glu Trp Leu Asp His Gly Glu Ala Gly Pro
370 375 380

Cys Ala Lys Gly Glu Gly Ala Pro Ser Asn Ile Val Gln Val Glu Pro
385 390 395 400

Phe Pro Glu Val Thr Tyr Thr Asn Leu Arg Trp Gly Glu Ile Gly Ser
405 410 415

Thr Tyr Gln Glu Val Gln Lys Pro Lys Pro Lys Pro Gly His Gly Pro
420 425 430

Arg Ser Asp
435

<210> 4
<211> 254
<212> PRT
<213> Humicola insolens

<400> 4

Met Leu Lys Ser Ala Leu Leu Leu Gly Pro Ala Ala Val Ser Val Gln
1 5 10 15

Ser Ala Ser Ile Pro Thr Ile Pro Ala Asn Leu Glu Pro Arg Gln Ile
20 25 30

Arg Ser Leu Cys Glu Leu Tyr Gly Tyr Trp Ser Gly Asn Gly Tyr Glu
35 40 45

Leu Leu Asn Asn Leu Trp Gly Lys Asp Thr Ala Thr Ser Gly Trp Gln
50 55 60

Cys Thr Tyr Leu Asp Gly Thr Asn Asn Gly Gly Ile Gln Trp Ser Thr
65 70 75 80

Ala Trp Glu Trp Gln Gly Ala Pro Asp Asn Val Lys Ser Tyr Pro Tyr
85 90 95

Val Gly Lys Gln Ile Gln Arg Gly Arg Lys Ile Ser Asp Ile Asn Ser
100 105 110

Met Arg Thr Ser Val Ser Trp Thr Tyr Asp Arg Thr Asp Ile Arg Ala
115 120 125

Asn Val Ala Tyr Asp Val Phe Thr Ala Arg Asp Pro Asp His Pro Asn
130 135 140

Trp Gly Gly Asp Tyr Glu Leu Met Ile Trp Leu Ala Arg Tyr Gly Gly
145 150 155 160

Ile Tyr Pro Ile Gly Thr Phe His Ser Gln Val Asn Leu Ala Gly Arg
165 170 175

Thr Trp Asp Leu Trp Thr Gly Tyr Asn Gly Asn Met Arg Val Tyr Ser
180 185 190

Phe Leu Pro Pro Ser Gly Asp Ile Arg Asp Phe Ser Cys Asp Ile Lys
195 200 205

Asp Phe Phe Asn Tyr Leu Glu Arg Asn His Gly Tyr Pro Ala Arg Glu
210 215 220

Gln Asn Leu Ile Val Tyr Gln Val Gly Thr Glu Cys Phe Thr Gly Gly
225 230 235 240

Pro Ala Arg Phe Thr Cys Arg Asp Phe Arg Ala Asp Leu Trp
245 250

<210> 5
<211> 388
<212> PRT
<213> Humicola insolens

<400> 5

Met Lys His Ser Val Leu Ala Gly Leu Phe Ala Thr Gly Ala Leu Ala
1 5 10 15

Gln Gly Gly Ala Trp Gln Gln Cys Gly Gly Val Gly Phe Ser Gly Ser
20 25 30

Thr Ser Cys Val Ser Gly Tyr Thr Cys Val Tyr Leu Asn Asp Trp Tyr
35 40 45

Ser Gln Cys Gln Pro Gln Pro Thr Thr Leu Arg Thr Thr Thr Thr Pro
50 55 60

Gly Ala Thr Ser Thr Thr Arg Ser Ala Pro Ala Ala Thr Ser Thr Thr
65 70 75 80

Pro Ala Lys Gly Lys Phe Lys Trp Phe Gly Ile Asn Gln Ser Cys Ala
85 90 95

Glu Phe Gly Lys Gly Glu Tyr Pro Gly Leu Trp Gly Lys His Phe Thr
100 105 110

Phe Pro Ser Thr Ser Ser Ile Gln Thr His Ile Asn Asp Gly Phe Asn
115 120 125

Met Phe Arg Val Ala Phe Ser Met Glu Arg Leu Ala Pro Asn Gln Leu
130 135 140

Asn Ala Ala Phe Asp Ala Asn Tyr Leu Arg Asn Leu Thr Glu Thr Val
145 150 155 160

Asn Phe Ile Thr Gly Lys Gly Lys Tyr Ala Met Leu Asp Pro His Asn

165 170 175

Phe Gly Arg Tyr Tyr Glu Arg Ile Ile Thr Asp Lys Ala Ala Phe Ala
180 185 190

Ser Phe Phe Thr Lys Leu Ala Thr His Phe Ala Ser Asn Pro Leu Val
195 200 205

Val Phe Asp Thr Asn Asn Glu Tyr His Asp Met Asp Gln Gln Leu Val
210 215 220

Phe Asp Leu Asn Gln Ala Ala Ile Asp Ala Ile Arg Ala Ala Gly Ala
225 230 235 240

Thr Ser Gln Tyr Ile Met Val Glu Gly Asn Ser Trp Thr Gly Ala Trp
245 250 255

Thr Trp Asn Val Thr Asn Asn Asn Leu Ala Ala Leu Arg Asp Pro Glu
260 265 270

Asn Lys Leu Val Tyr Gln Met His Gln Tyr Leu Asp Ser Asp Gly Ser
275 280 285

Gly Thr Ser Thr Ala Cys Val Ser Thr Gln Val Gly Leu Gln Arg Val
290 295 300

Ile Gly Ala Thr Asn Trp Leu Arg Gln Asn Gly Lys Val Gly Leu Leu
305 310 315 320

Gly Glu Phe Ala Gly Gly Ala Asn Ser Val Cys Gln Gln Ala Ile Glu
325 330 335

Gly Met Leu Thr His Leu Gln Glu Asn Ser Asp Val Trp Thr Gly Ala
340 345 350

Leu Trp Trp Ala Gly Gly Pro Trp Trp Gly Asp Tyr Ile Tyr Ser Phe
355 360 365

Glu Pro Pro Ser Gly Ile Gly Tyr Thr Tyr Tyr Asn Ser Leu Leu Lys
370 375 380

Lys Tyr Val Pro
385

<210> 6
<211> 305
<212> PRT
<213> Humicola insolens

<400> 6

Met Arg Ser Ser Pro Leu Leu Arg Ser Ala Val Val Ala Ala Leu Pro
1 5 10 15

Val Leu Ala Leu Ala Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Cys
20 25 30

Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro
35 40 45

Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala
50 55 60

Lys Ser Gly Cys Glu Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln
65 70 75 80

Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr
85 90 95

Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu
100 105 110

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Val Val Gln
115 120 125

Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn
130 135 140

Ile Pro Gly Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe
145 150 155 160

Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu
165 170 175

Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe
180 185 190

Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val
195 200 205

Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp
210 215 220

Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser Thr Ser Ser
225 230 235 240

Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr Ser Thr Ser Thr Thr
245 250 255

Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys Thr Ala Glu
260 265 270

Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys Thr Thr Cys
275 280 285

Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr His Gln Cys
290 295 300

Leu
305

<210> 7
<211> 335
<212> PRT
<213> Thermoascus aurantiacus

<400> 7

Met Lys Leu Gly Ser Leu Val Leu Ala Leu Ser Ala Ala Arg Leu Thr
1 5 10 15

Leu Ser Ala Pro Leu Ala Asp Arg Lys Gln Glu Thr Lys Arg Ala Lys
20 25 30

Val Phe Gln Trp Phe Gly Ser Asn Glu Ser Gly Ala Glu Phe Gly Ser
35 40 45

Gln Asn Leu Pro Gly Val Glu Gly Lys Asp Tyr Ile Trp Pro Asp Pro
50 55 60

Asn Thr Ile Asp Thr Leu Ile Ser Lys Gly Met Asn Ile Phe Arg Val
65 70 75 80

Pro Phe Met Met Glu Arg Leu Val Pro Asn Ser Met Thr Gly Ser Pro
85 90 95

Asp Pro Asn Tyr Leu Ala Asp Leu Ile Ala Thr Val Asn Ala Ile Thr
100 105 110

Gln Lys Gly Ala Tyr Ala Val Val Asp Pro His Asn Tyr Gly Arg Tyr
115 120 125

Tyr Asn Ser Ile Ile Ser Ser Pro Ser Asp Phe Gln Thr Phe Trp Lys
130 135 140

Thr Val Ala Ser Gln Phe Ala Ser Asn Pro Leu Val Ile Phe Asp Thr
145 150 155 160

Asn Asn Glu Tyr His Asp Met Asp Gln Thr Leu Val Leu Asn Leu Asn
165 170 175

Gln Ala Ala Ile Asp Gly Ile Arg Ser Ala Gly Ala Thr Ser Gln Tyr
180 185 190

Ile Phe Val Glu Gly Asn Ser Trp Thr Gly Ala Trp Thr Trp Thr Asn
195 200 205

Val Asn Asp Asn Met Lys Ser Leu Thr Asp Pro Ser Asp Lys Ile Ile
    210   215   220

Tyr Glu Met His Gln Tyr Leu Asp Ser Asp Gly Ser Gly Thr Ser Ala
225   230   235   240

Thr Cys Val Ser Ser Thr Ile Gly Gln Glu Arg Ile Thr Ser Ala Thr
                245   250   255

Gln Trp Leu Arg Ala Asn Gly Lys Lys Gly Ile Ile Gly Glu Phe Ala
            260   265   270

Gly Gly Ala Asn Asp Val Cys Glu Thr Ala Ile Thr Gly Met Leu Asp
        275   280   285

Tyr Met Ala Gln Asn Thr Asp Val Trp Thr Gly Ala Ile Trp Trp Ala
    290   295   300

Ala Gly Pro Trp Trp Gly Asp Tyr Ile Phe Ser Met Glu Pro Asp Asn
305   310   315   320

Gly Ile Ala Tyr Gln Gln Ile Leu Pro Ile Leu Thr Pro Tyr Leu
                325   330   335

<210> 8
<211> 327
<212> PRT
<213> Aspergillus aculeatus

<400> 8

Met Val Gln Ile Lys Ala Ala Ala Leu Ala Val Leu Phe Ala Ser Asn
1   5   10   15

Val Leu Ser Asn Pro Ile Glu Pro Arg Gln Ala Ser Val Ser Ile Asp
            20   25   30

Ala Lys Phe Lys Ala His Gly Lys Lys Tyr Leu Gly Thr Ile Gly Asp
        35   40   45

Gln Tyr Thr Leu Asn Lys Asn Ala Lys Thr Pro Ala Ile Ile Lys Ala
    50   55   60

Asp Phe Gly Gln Leu Thr Pro Glu Asn Ser Met Lys Trp Asp Ala Thr
65   70   75   80

Glu Pro Asn Arg Gly Gln Phe Ser Phe Ser Gly Ser Asp Tyr Leu Val
                85   90   95

Asn Phe Ala Gln Ser Asn Gly Lys Leu Ile Arg Gly His Thr Leu Val
            100   105   110

```
Trp His Ser Gln Leu Pro Ser Trp Val Gln Ser Ile Ser Asp Lys Asn
        115                 120                 125

Thr Leu Ile Gln Val Met Gln Asn His Ile Thr Thr Val Met Gln Arg
    130                 135                 140

Tyr Lys Gly Lys Val Tyr Ala Trp Asp Val Val Asn Glu Ile Phe Asn
145                 150                 155                 160

Glu Asp Gly Ser Leu Cys Gln Ser His Phe Tyr Asn Val Ile Gly Glu
                165                 170                 175

Asp Tyr Val Arg Ile Ala Phe Glu Thr Ala Arg Ala Val Asp Pro Asn
            180                 185                 190

Ala Lys Leu Tyr Ile Asn Asp Tyr Asn Leu Asp Ser Ala Ser Tyr Pro
        195                 200                 205

Lys Leu Thr Gly Leu Val Asn His Val Lys Lys Trp Val Ala Ala Gly
    210                 215                 220

Val Pro Ile Asp Gly Ile Gly Ser Gln Thr His Leu Ser Ala Gly Ala
225                 230                 235                 240

Gly Ala Ala Val Ser Gly Ala Leu Asn Ala Leu Ala Gly Ala Gly Thr
                245                 250                 255

Lys Glu Val Ala Ile Thr Glu Leu Asp Ile Ala Gly Ala Ser Ser Thr
            260                 265                 270

Asp Tyr Val Asn Val Val Lys Ala Cys Leu Asn Gln Pro Lys Cys Val
        275                 280                 285

Gly Ile Thr Val Trp Gly Ser Ser Asp Pro Asp Ser Trp Arg Ser Ser
    290                 295                 300

Ser Ser Pro Leu Leu Phe Asp Ser Asn Tyr Asn Pro Lys Ala Ala Tyr
305                 310                 315                 320

Thr Ala Ile Ala Asn Ala Leu
                325
```

<210> 9
<211> 406
<212> PRT
<213> Aspergillus aculeatus

<400> 9

```
Met Val Gly Leu Leu Ser Ile Thr Ala Ala Leu Ala Ala Thr Val Leu
1               5                   10                  15

Pro Asn Ile Val Ser Ala Val Gly Leu Asp Gln Ala Ala Val Ala Lys
```

20 25 30

Gly Leu Gln Tyr Phe Gly Thr Ala Thr Asp Asn Pro Glu Leu Thr Asp
35 40 45

Ile Pro Tyr Val Thr Gln Leu Asn Asn Thr Ala Asp Phe Gly Gln Ile
50 55 60

Thr Pro Gly Asn Ser Met Lys Trp Asp Ala Thr Glu Pro Ser Gln Gly
65 70 75 80

Thr Phe Thr Phe Thr Lys Gly Asp Val Ile Ala Asp Leu Ala Glu Gly
85 90 95

Asn Gly Gln Tyr Leu Arg Cys His Thr Leu Val Trp Tyr Asn Gln Leu
100 105 110

Pro Ser Trp Val Thr Ser Gly Thr Trp Thr Asn Ala Thr Leu Thr Ala
115 120 125

Ala Leu Lys Asn His Ile Thr Asn Val Val Ser His Tyr Lys Gly Lys
130 135 140

Cys Leu His Trp Asp Val Val Asn Glu Ala Leu Asn Asp Asp Gly Thr
145 150 155 160

Tyr Arg Thr Asn Ile Phe Tyr Thr Thr Ile Gly Glu Ala Tyr Ile Pro
165 170 175

Ile Ala Phe Ala Ala Ala Ala Ala Ala Asp Pro Asp Ala Lys Leu Phe
180 185 190

Tyr Asn Asp Tyr Asn Leu Glu Tyr Gly Gly Ala Lys Ala Ala Ser Ala
195 200 205

Arg Ala Ile Val Gln Leu Val Lys Asn Ala Gly Ala Lys Ile Asp Gly
210 215 220

Val Gly Leu Gln Ala His Phe Ser Val Gly Thr Val Pro Ser Thr Ser
225 230 235 240

Ser Leu Val Ser Val Leu Gln Ser Phe Thr Ala Leu Gly Val Glu Val
245 250 255

Ala Tyr Thr Glu Ala Asp Val Arg Ile Leu Leu Pro Thr Thr Ala Thr
260 265 270

Thr Leu Ala Gln Gln Ser Ser Asp Phe Gln Ala Leu Val Gln Ser Cys
275 280 285

Val Gln Thr Thr Gly Cys Val Gly Phe Thr Ile Trp Asp Trp Thr Asp

290 295 300

Lys Tyr Ser Trp Val Pro Ser Thr Phe Ser Gly Tyr Gly Ala Ala Leu
305 310 315 320

Pro Trp Asp Glu Asn Leu Val Lys Lys Pro Ala Tyr Asn Gly Leu Leu
325 330 335

Ala Gly Met Gly Val Thr Val Thr Thr Thr Thr Thr Thr Thr Thr Ala
340 345 350

Thr Ala Thr Gly Lys Thr Thr Thr Thr Thr Thr Gly Ala Thr Ser Thr
355 360 365

Gly Thr Thr Ala Ala His Trp Gly Gln Cys Gly Gly Leu Asn Trp Ser
370 375 380

Gly Pro Thr Ala Cys Ala Thr Gly Tyr Thr Cys Thr Tyr Val Asn Asp
385 390 395 400

Tyr Tyr Ser Gln Cys Leu
405

<210> 10
<211> 231
<212> PRT
<213> Aspergillus aculeatus

<400> 10

Met Ala Arg Leu Ser Gln Phe Leu Leu Ala Cys Ala Leu Ala Val Lys
1 5 10 15

Ala Phe Ala Ala Pro Ala Ala Glu Pro Val Glu Glu Arg Gly Pro Asn
20 25 30

Phe Phe Ser Ala Leu Ala Gly Arg Ser Thr Gly Ser Ser Thr Gly Tyr
35 40 45

Ser Asn Gly Tyr Tyr Tyr Ser Phe Trp Thr Asp Gly Ala Ser Gly Asp
50 55 60

Val Glu Tyr Ser Asn Gly Ala Gly Gly Ser Tyr Ser Val Thr Trp Ser
65 70 75 80

Ser Ala Ser Asn Phe Val Gly Gly Lys Gly Trp Asn Pro Gly Ser Ala
85 90 95

His Asp Ile Thr Tyr Ser Gly Ser Trp Thr Ser Thr Gly Asn Ser Asn
100 105 110

Ser Tyr Leu Ser Val Tyr Gly Trp Thr Thr Gly Pro Leu Val Glu Tyr
115 120 125

Tyr Ile Leu Glu Asp Tyr Gly Glu Tyr Asn Pro Gly Ser Ala Gly Thr
130 135 140

Tyr Lys Gly Ser Val Tyr Ser Asp Gly Ser Thr Tyr Asn Ile Tyr Thr
145 150 155 160

Ala Thr Arg Thr Asn Ala Pro Ser Ile Gln Gly Thr Ala Thr Phe Thr
165 170 175

Gln Tyr Trp Ser Ile Arg Gln Thr Lys Arg Val Gly Gly Thr Val Thr
180 185 190

Thr Ala Asn His Phe Asn Ala Trp Ala Lys Leu Gly Met Asn Leu Gly
195 200 205

Thr His Asn Tyr Gln Ile Val Ala Thr Glu Gly Tyr Tyr Ser Ser Gly
210 215 220

Ser Ala Ser Ile Thr Val Ala
225 230

<210> 11
<211> 227
<212> PRT
<213> Humicola insolens

<400> 11

Met Val Ser Leu Lys Ser Val Leu Ala Ala Ala Thr Ala Val Ser Ser
1 5 10 15

Ala Ile Ala Ala Pro Phe Asp Phe Val Pro Arg Asp Asn Ser Thr Ala
20 25 30

Leu Gln Ala Arg Gln Val Thr Pro Asn Ala Glu Gly Trp His Asn Gly
35 40 45

Tyr Phe Tyr Ser Trp Trp Ser Asp Gly Gly Gly Gln Val Gln Tyr Thr
50 55 60

Asn Leu Glu Gly Ser Arg Tyr Gln Val Arg Trp Arg Asn Thr Gly Asn
65 70 75 80

Phe Val Gly Gly Lys Gly Trp Asn Pro Gly Thr Gly Arg Thr Ile Asn
85 90 95

Tyr Gly Gly Tyr Phe Asn Pro Gln Gly Asn Gly Tyr Leu Ala Val Tyr
100 105 110

Gly Trp Thr Arg Asn Pro Leu Val Glu Tyr Tyr Val Ile Glu Ser Tyr
115 120 125

Gly Thr Tyr Asn Pro Gly Ser Gln Ala Gln Tyr Lys Gly Thr Phe Tyr
130 135 140

Thr Asp Gly Asp Gln Tyr Asp Ile Phe Val Ser Thr Arg Tyr Asn Gln
145 150 155 160

Pro Ser Ile Asp Gly Thr Arg Thr Phe Gln Gln Tyr Trp Ser Ile Arg
165 170 175

Lys Asn Lys Arg Val Gly Gly Ser Val Asn Met Gln Asn His Phe Asn
180 185 190

Ala Trp Gln Gln His Gly Met Pro Leu Gly Gln His Tyr Tyr Gln Val
195 200 205

Val Ala Thr Glu Gly Tyr Gln Ser Ser Gly Glu Ser Asp Ile Tyr Val
210 215 220

Gln Thr His
225

<210> 12
<211> 389
<212> PRT
<213> Humicola insolens

<400> 12

Met Arg Ser Ile Ala Leu Ala Leu Ala Ala Ala Pro Ala Val Leu Ala
1 5 10 15

Gln Ser Gln Leu Trp Gly Gln Cys Gly Gly Ile Gly Trp Asn Gly Pro
20 25 30

Thr Thr Cys Val Ser Gly Ala Thr Cys Thr Lys Ile Asn Asp Trp Tyr
35 40 45

His Gln Cys Leu Pro Gly Gly Asn Asn Asn Asn Pro Pro Pro Ala Thr
50 55 60

Thr Ser Gln Trp Thr Pro Pro Pro Ala Gln Thr Ser Ser Asn Pro Pro
65 70 75 80

Pro Thr Gly Gly Gly Gly Gly Asn Thr Leu His Glu Lys Phe Lys Ala
85 90 95

Arg Gly Lys Gln Tyr Phe Gly Thr Glu Ile Asp His Tyr His Leu Asn
100 105 110

Asn Asn Gln Leu Met Glu Ile Ala Arg Arg Glu Phe Gly Gln Ile Thr
115 120 125

His Glu Asn Ser Met Lys Trp Asp Ala Thr Glu Pro Ser Arg Gly Ser
130 135 140

Phe Ser Phe Gly Asn Ala Asp Arg Val Val Asp Trp Ala Thr Ser Asn
145 150 155 160

Gly Lys Leu Ile Arg Gly His Thr Leu Leu Trp His Ser Gln Leu Pro
165 170 175

Gln Trp Val Gln Asn Ile Asn Asp Arg Asn Thr Leu Thr Gln Val Ile
180 185 190

Glu Asn His Val Arg Thr Val Met Thr Arg Tyr Lys Gly Lys Ile Phe
195 200 205

His Tyr Asp Val Val Asn Glu Ile Leu Asp Glu Asn Gly Gly Leu Arg
210 215 220

Asn Ser Val Phe Ser Arg Val Leu Gly Glu Asp Phe Val Gly Ile Ala
225 230 235 240

Phe Arg Ala Ala Arg Ala Ala Asp Pro Asp Ala Lys Leu Tyr Ile Asn
245 250 255

Asp Tyr Asn Leu Asp Ser Ala Asn Tyr Ala Lys Thr Arg Gly Met Ile
260 265 270

Asn Leu Val Asn Lys Trp Val Ser Gln Gly Val Pro Ile Asp Gly Ile
275 280 285

Gly Thr Gln Ala His Leu Ala Gly Pro Gly Gly Trp Asn Pro Ala Ser
290 295 300

Gly Val Pro Ala Ala Leu Gln Ala Leu Ala Gly Ala Asn Val Lys Glu
305 310 315 320

Val Ala Ile Thr Glu Leu Asp Ile Gln Gly Ala Gly Ala Asn Asp Tyr
325 330 335

Val Thr Val Ala Asn Ala Cys Leu Asn Val Gln Lys Cys Val Gly Ile
340 345 350

Thr Val Trp Gly Val Ser Asp Arg Asp Thr Trp Arg Ser Asn Glu Asn
355 360 365

Pro Leu Leu Tyr Asp Arg Asp Tyr Arg Pro Lys Ala Ala Tyr Asn Ala
370 375 380

Leu Met Asn Ala Leu
385

<210> 13
<211> 375
<212> PRT
<213> Myceliophthora thermophila

<400> 13

Met His Leu Ser Ser Ser Leu Leu Leu Leu Ala Ala Leu Pro Leu Gly
1 5 10 15

Ile Ala Gly Lys Gly Lys Gly His Gly His Gly Pro His Thr Gly Leu
20 25 30

His Thr Leu Ala Lys Gln Ala Gly Leu Lys Tyr Phe Gly Ser Ala Thr
35 40 45

Asp Ser Pro Gly Gln Arg Glu Arg Ala Gly Tyr Glu Asp Lys Tyr Ala
50 55 60

Gln Tyr Asp Gln Ile Met Trp Lys Ser Gly Glu Phe Gly Leu Thr Thr
65 70 75 80

Pro Thr Asn Gly Gln Lys Trp Leu Phe Thr Glu Pro Glu Arg Gly Val
85 90 95

Phe Asn Phe Thr Glu Gly Asp Ile Val Thr Asn Leu Ala Arg Lys His
100 105 110

Gly Phe Met Gln Arg Cys His Ala Leu Val Trp His Ser Gln Leu Ala
115 120 125

Pro Trp Val Glu Ser Thr Glu Trp Thr Pro Glu Glu Leu Arg Gln Val
130 135 140

Ile Val Asn His Ile Thr His Val Ala Gly Tyr Tyr Lys Gly Lys Cys
145 150 155 160

Tyr Ala Trp Asp Val Val Asn Glu Ala Leu Asn Glu Asp Gly Thr Tyr
165 170 175

Arg Glu Ser Val Phe Tyr Lys Val Leu Gly Glu Asp Tyr Ile Lys Leu
180 185 190

Ala Phe Glu Thr Ala Ala Lys Val Asp Pro His Ala Lys Leu Tyr Tyr
195 200 205

Asn Asp Tyr Asn Leu Glu Ser Pro Ser Ala Lys Thr Glu Gly Ala Lys
210 215 220

Arg Ile Val Lys Met Leu Lys Asp Ala Gly Ile Arg Ile Asp Gly Val
225 230 235 240

Gly Leu Gln Ala His Leu Val Ala Glu Ser His Pro Thr Leu Asp Glu

245 250 255

His Ile Asp Ala Ile Lys Gly Phe Thr Glu Leu Gly Val Glu Val Ala
260 265 270

Leu Thr Glu Leu Asp Ile Arg Leu Ser Ile Pro Ala Asn Ala Thr Asn
275 280 285

Leu Ala Gln Gln Arg Glu Ala Tyr Lys Asn Val Val Gly Ala Cys Val
290 295 300

Gln Val Arg Gly Cys Ile Gly Val Glu Ile Trp Asp Phe Tyr Asp Pro
305 310 315 320

Phe Ser Trp Val Pro Ala Thr Phe Pro Gly Gln Gly Ala Pro Leu Leu
325 330 335

Trp Phe Glu Asp Phe Ser Lys His Pro Ala Tyr Asp Gly Val Val Glu
340 345 350

Ala Leu Thr Asn Arg Thr Thr Gly Gly Cys Lys Gly Lys Gly Lys Gly
355 360 365

Lys Gly Lys Val Trp Lys Ala
370 375

<210> 14
<211> 226
<212> PRT
<213> Myceliophthora thermophila

<400> 14

Met Val Thr Leu Thr Arg Leu Ala Val Ala Ala Ala Ala Met Ile Ser
1 5 10 15

Ser Thr Gly Leu Ala Ala Pro Thr Pro Glu Ala Gly Pro Asp Leu Pro
20 25 30

Asp Phe Glu Leu Gly Val Asn Asn Leu Ala Arg Arg Ala Leu Asp Tyr
35 40 45

Asn Gln Asn Tyr Arg Thr Ser Gly Asn Val Asn Tyr Ser Pro Thr Asp
50 55 60

Asn Gly Tyr Ser Val Ser Phe Ser Asn Ala Gly Asp Phe Val Val Gly
65 70 75 80

Lys Gly Trp Arg Thr Gly Ala Thr Arg Asn Ile Thr Phe Ser Gly Ser
85 90 95

Thr Gln His Thr Ser Gly Thr Val Leu Val Ser Val Tyr Gly Trp Thr
100 105 110

Arg Asn Pro Leu Ile Glu Tyr Tyr Val Gln Glu Tyr Thr Ser Asn Gly
115 120 125

Ala Gly Ser Ala Gln Gly Glu Lys Leu Gly Thr Val Glu Ser Asp Gly
130 135 140

Gly Thr Tyr Glu Ile Trp Arg His Gln Gln Val Asn Gln Pro Ser Ile
145 150 155 160

Glu Gly Thr Ser Thr Phe Trp Gln Tyr Ile Ser Asn Arg Val Ser Gly
165 170 175

Gln Arg Pro Asn Gly Gly Thr Val Thr Leu Ala Asn His Phe Ala Ala
180 185 190

Trp Gln Lys Leu Gly Leu Asn Leu Gly Gln His Asp Tyr Gln Val Leu
195 200 205

Ala Thr Glu Gly Trp Gly Asn Ala Gly Gly Ser Ser Gln Tyr Thr Val
210 215 220

Ser Gly
225

<210> 15
<211> 225
<212> PRT
<213> Thermomyces lanuginosus

<400> 15

Met Val Gly Phe Thr Pro Val Ala Leu Ala Ala Leu Ala Ala Thr Gly
1 5 10 15

Ala Leu Ala Phe Pro Ala Gly Asn Ala Thr Glu Leu Glu Lys Arg Gln
20 25 30

Thr Thr Pro Asn Ser Glu Gly Trp His Asp Gly Tyr Tyr Tyr Ser Trp
35 40 45

Trp Ser Asp Gly Gly Ala Gln Ala Thr Tyr Thr Asn Leu Glu Gly Gly
50 55 60

Thr Tyr Glu Ile Ser Trp Gly Asp Gly Gly Asn Leu Val Gly Gly Lys
65 70 75 80

Gly Trp Asn Pro Gly Leu Asn Ala Arg Ala Ile His Phe Glu Gly Val
85 90 95

Tyr Gln Pro Asn Gly Asn Ser Tyr Leu Ala Val Tyr Gly Trp Thr Arg
100 105 110

```
Asn Pro Leu Val Glu Tyr Tyr Ile Val Glu Asn Phe Gly Thr Tyr Asp
        115                 120                 125

Pro Ser Ser Gly Ala Thr Asp Leu Gly Thr Val Glu Cys Asp Gly Ser
    130                 135                 140

Ile Tyr Arg Leu Gly Lys Thr Thr Arg Val Asn Ala Pro Ser Ile Asp
145                 150                 155                 160

Gly Thr Gln Thr Phe Asp Gln Tyr Trp Ser Val Arg Gln Asp Lys Arg
                165                 170                 175

Thr Ser Gly Thr Val Gln Thr Gly Cys His Phe Asp Ala Trp Ala Arg
            180                 185                 190

Ala Gly Leu Asn Val Asn Gly Asp His Tyr Tyr Gln Ile Val Ala Thr
        195                 200                 205

Glu Gly Tyr Phe Ser Ser Gly Tyr Ala Arg Ile Thr Val Ala Asp Val
    210                 215                 220

Gly
225

<210>  16
<211>  237
<212>  PRT
<213>  Aspergillus aculeatus

<400>  16

Met Lys Ala Phe Tyr Phe Leu Ala Ser Leu Ala Gly Ala Ala Val Ala
1               5                   10                  15

Gln Gln Thr Gln Leu Cys Asp Gln Tyr Ala Thr Tyr Thr Gly Ser Val
            20                  25                  30

Tyr Thr Ile Asn Asn Asn Leu Trp Gly Lys Asp Ala Gly Ser Gly Ser
        35                  40                  45

Gln Cys Thr Thr Val Asn Ser Ala Ser Ser Ala Gly Thr Ser Trp Ser
    50                  55                  60

Thr Lys Trp Asn Trp Ser Gly Gly Glu Asn Ser Val Lys Ser Tyr Ala
65                  70                  75                  80

Asn Ser Gly Leu Ser Phe Asn Lys Lys Leu Val Ser Gln Ile Ser Arg
                85                  90                  95

Ile Pro Thr Ala Ala Gln Trp Ser Tyr Asp Asn Thr Gly Ile Arg Ala
            100                 105                 110
```

```
Asp Val Ala Tyr Asp Leu Phe Thr Ala Ala Asp Ile Asn His Val Thr
        115                 120                 125

Trp Ser Gly Asp Tyr Glu Leu Met Ile Trp Leu Ala Arg Tyr Gly Gly
    130                 135                 140

Val Gln Pro Leu Gly Ser Lys Ile Ala Thr Ala Thr Val Glu Gly Gln
145                 150                 155                 160

Thr Trp Glu Leu Trp Tyr Gly Val Asn Gly Ala Gln Lys Thr Tyr Ser
                165                 170                 175

Phe Val Ala Pro Thr Pro Ile Thr Ser Phe Gln Gly Asp Val Asn Asp
            180                 185                 190

Phe Phe Lys Tyr Leu Thr Gln Asn His Gly Phe Pro Ala Ser Ser Gln
        195                 200                 205

Tyr Leu Ile Thr Leu Gln Phe Gly Thr Glu Pro Phe Thr Gly Gly Pro
    210                 215                 220

Ala Thr Leu Thr Val Ser Asp Trp Ser Ala Ser Val Gln
225                 230                 235
```